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THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

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VITAMIN A IN POULTRY.
Effects of Vitamin Deficiency.

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SUMMARY.

The results of two trials to determine the effects of vitamin A deficiency on fowls and on their progeny are presented.

In the 1955 pilot trial, hens receiving a ration containing no preformed vitamin A and no measurable quantity of carotene were not depleted of vitamin A in over seven months. This was due to their high initial reserves.

In the 1956 trial, pullets were used and artificial insemination and trap-nesting introduced.

The egg production of the vitamin A deficient groups was 23 per cent. lower than that of the vitamin A adequate controls when the deficiency was first established by liver analyses, 57 per cent. lower when lesions of the deficiency were first apparent by field examination, and finally production ceased. Egg production of pullets that were reversed after field evidence of vitamin A deficiency was established returned to normal two weeks after supplementation with vitamin A.

The hatchability of eggs of the vitamin A deficient groups was 40–50 per cent. when the deficiency was first established by liver analyses, 10–20 per cent. when field evidence of vitamin A deficiency was established, and finally nil. The average hatchability of the vitamin A adequate controls was 80 per cent. On reversal, the hatchability returned to normal one week after supplementation with vitamin A.

Vitamin A deficiency affects hatchability of eggs before it affects egg production.

On the vitamin A deficient ration, a decrease in egg production and hatchability was also associated with marginal liver vitamin A reserves.

When vitamin A deficiency was first confirmed, the eggs laid contained less than 8 μg. of vitamin A per yolk compared with 42 μg. from the controls.

There was no evidence of reduced hatchability due to vitamin A deficiency when total yolk-sac and liver reserves of vitamin A in day-old chickens were in excess of 8 μg.

Nodules on the oesophagus, although present on field examination, were not apparent in four pullets at autopsy; this was due to post-mortem decomposition. The disappearance of nodules after vitamin A supplementation may take up to eight weeks.

Pullets on a vitamin A deficient ration survived for a maximum period of 39½ weeks after the deficiency had been established by chemical analyses. Of the 24 deaths in the deficient groups, 10 were due to uncomplicated vitamin A deficiency, 5 to visceral gout, 4 to a rupture of the liver, 3 to egg peritonitis, 1 to myocardial degeneration and 1 pullet was too decomposed to show any lesions. No deaths occurred prior to the onset of vitamin A deficiency.

There was no difference in food consumption between groups, and pullets that succumbed to vitamin A deficiency were not emaciated.

Chickens were reared up to four weeks of age in four consecutive hatches from eggs of vitamin A sufficient and deficient pullets.

Rearing mortality was 71 per cent. in progeny from deficient pullets when reared on a deficient ration, 41 per cent. in progeny from deficient pullets when reared on a sufficient ration, 17 per cent. in progeny from deficient pullets when reared on a heavily supplemented ration (5x sufficient), 25 per cent. in progeny from sufficient pullets when reared on a deficient ration, and 4 per cent. in progeny from sufficient pullets when reared on a sufficient ration. Most deaths occurred towards the end of the first week of life.

I. INTRODUCTION.

The available literature contains numerous references to the effects of vitamin A deficiency on fowls of all ages. Early workers presented results which were not always in agreement and which were not usually supported by chemical analyses. Often carotene-containing feedstuffs were used as the sole source of vitamin A. Thus differences, attributed to vitamin A, between groups which were on a basal ration without green-feed and a basal ration plus green-feed could have been due at least partly to the effect of factors additional to carotene in the green-feed.

Polk and Sipe (1940), in their work on the effect of vitamin A deficiency on malposition of the chick embryo, used a depletion ration incorporating a small amount of carotene. Their figures, when subjected to further analysis, indicate that hatchability was unaffected by the level of vitamin A in the rations, being of the order of about 50 per cent. in both groups, with a high percentage of infertile eggs. Egg production was 27 per cent. less in the group on the depletion ration.

Rubin and Bird (1942) studied the progressive effects of vitamin A deficiency in laying fowls. Carotene was the sole source of vitamin A in the control group. They found that although egg production was decreased to almost zero, there was no appreciable effect on hatchability.

Temperton and Dudley (1946) placed four groups of fowls on a vitamin A depletion diet to study the effects of progressive vitamin A depletion in adult fowls. After one month some fowls developed clinical symptoms of vitamin A deficiency and all groups were then allowed access to runs of poor grass. The experiment did not yield clear-cut partitioning of the progressive stages of vitamin depletion. Fertility was unaffected by a deficiency in vitamin A, but a decline in hatchability occurred after the groups were taken off the grass runs and confined in pens. A decline in egg production was also apparent at this stage and was concurrent with clinical cases of vitamin A deficiency. Although these effects were probably due to vitamin A deficiency, the fall in hatchability could have been due to an inadequate intake of riboflavin after the fowls were taken off the grass runs. The ration used contributed only

1,185 micrograms riboflavin per lb. of feed, whereas Ewing (1947) recommended for satisfactory hatchability a minimum of 1,300 micrograms of riboflavin per lb. of feed for breeding hens.

Taylor, Stern, Russell and Jungherr (1947) conducted experiments to determine the provitamin A requirements of hens. They controlled the vitamin A potency of their rations by using different types and amounts of carotene-containing feeds. In comparing two groups for egg production, a drop in one was believed to be significant and to be due to a lower intake of carotene. The roosters in the respective groups were apparently maintained on the same rations as the hens, and a lowered hatchability in the group on the lowest carotene level was accentuated by an increased number of infertile eggs.

McClymont and Hart (1948) investigated the effect of an inadequate intake of vitamin A on egg production and hatchability. Their control ration contained green-feed throughout the experiment. Their deficient ration contained fish oil for the first six weeks to provide an adequate reserve of vitamin A in the fowls of this group. After six weeks, fish oil was omitted from their deficient ration. They found that vitamin A deficiency resulted in a cessation of egg production not preceded by a marked decline. There was no apparent effect on the fertility and hatchability of eggs. Some of the fowls on the deficient ration died.

Heywang (1952) obtained information on the level of vitamin A in the diet of laying and breeding fowls during hot weather. He used vitamin A *per se* to supplement the feed of six groups of pullets at varying levels. Data on egg production and hatchability at the commencement of the experiment and after 84 days indicated that the pullets on the unsupplemented ration laid fewer eggs, and these were of lower hatchability. No vitamin A analyses were presented.

A survey of the vitamin A reserves in fowls by Gartner and Ryley (1956) indicated that vitamin A deficiency is still a major cause of economic loss in commercial flocks in Queensland, but in this survey the vitamin A reserves of the fowls were related to diseases rather than to production. The object of the investigation reported here was to determine the effects of vitamin A deficiency in laying fowls and response from vitamin A supplementation once field evidence of vitamin A deficiency had been established. At various stages of the experiment chemical analyses were made on the livers of hens and their progeny, on eggs, on the feed and in one instance on faeces. Chemical data were obtained at certain stages to aid the evaluation of marginal vitamin A status in adult fowls in relation to egg production and hatchability and in chickens in relation to the vitamin A status of their dams. The results are presented in this paper.

II. METHODS.

(1) Husbandry in 1955 Pilot Trial.

A pilot trial was initiated on June 1, 1955, to determine whether feeding a vitamin A deficient ration to laying fowls would lower the hatchability of eggs and, if so, at what rate. In addition, data on the effectiveness of fertilisation by roosters rotated between pens was obtained.

The only hens available at the time were 18 months' old White Leghorns and White Leghorn–Australorp crossbreds. They were divided at random into two groups each containing 22 hens. These hens were housed in adjacent intensive pens, each pen measuring 14 ft. x 14 ft. They were run on deep litter consisting of pine shavings, which remained in the pens for the duration of the trial.

A male bird was placed in each pen. At first the males were interchanged twice a week to equalise their individual variations and their intake of vitamin A, but later were interchanged every second day owing to poor fertility in the group on the vitamin A deficient ration. Fertility did not improve in this group and for the last four hatches of the trial artificial insemination was carried out, using Australorp males.

The basal ration fed to one group contained no preformed vitamin A and no measurable quantity of carotene. The other group was fed this basal ration plus stabilized vitamin A (10,000 I.U./g.), incorporated to provide a ration containing about 2,800 I.U. of vitamin A per lb.

*Composition of Basal Ration.**

	lb.
Sorghum Meal	25
Wheatmeal	15
Bran	27
Pollard	23
Meatmeal	9½
Salt Premix +	½
	100
Vitamin D_3	280 I.U./lb.

+ Salt Premix consisted of 10 lb. salt to which were added 80 g. manganese sulphate and 2.6 g. synthetic riboflavin.

* The total riboflavin content of the ration was 2.8 mg./lb. feed.

The rations were fed *ad lib.* in open feed hoppers. Shellgrit and insoluble grit were made freely available. The ration was analysed for riboflavin initially and for vitamin A periodically. For the purpose of this paper, the basal ration will be referred to as the deficient ration and the basal ration plus added vitamin A (2,800 I.U./g.) as the sufficient ration.

Weekly egg production per group was recorded and suitable eggs incubated weekly to determine the hatchability. All eggs set were of good shell texture, were free from any cracks and had a minimum weight of $1\frac{7}{8}$ oz. Thin-shelled eggs and oversized eggs were not set. No consideration was paid to the shape of the egg. The eggs were set in a forced-draught incubator and the position of each tray was alternated weekly. Fumigation was carried out on the 18th day, when the eggs had been transferred to the hatching compartment.

At intervals, eggs and newly-hatched chickens were analysed for vitamin A. The livers of hens that died during the trial and of hens killed to maintain equal numbers in each group were also analysed. The trial was terminated on Jan. 20, 1956, and the livers of five hens from the control group and four from the group on the vitamin A deficient ration were analysed.

(2) Husbandry in 1956 Trial.

The second trial was planned on experience gained in the pilot trial. A group of Australorp pullets, 18 weeks of age, was taken. At five days of age they had been vaccinated with pigeon pox vaccine. From four to 10 weeks of age, sulphaquinoxaline (·0125 per cent.) had been incorporated in the mash as a preventive against coccidiosis.

Prior to being selected for the experiment, the pullets had been fed a diet high in vitamin A. Two pullets were killed and analyses confirmed that initial liver reserves of vitamin A were high. All the pullets were placed for 74 days on the vitamin A deficient ration used in the pilot trial. At the end of this time a further two were killed. These had liver reserves comparable with average levels encountered in practice. The pullets were divided into two groups at random on Apr. 17, 1956, and each group of 36 placed in separate but adjacent intensive pens. One group was maintained on the vitamin A deficient ration and will be referred to as the deficient group (D Group). The other group was placed on the vitamin A sufficient ration used in the pilot trial and will be referred to as the sufficient group (S Group).

The pullets were trap-nested and individual records on production and hatchability were kept. All the pullets were artificially inseminated twice a week for the first two weeks and then once weekly thereafter with pooled semen from four Australorp roosters fed a ration containing the same amount of stabilized vitamin A as was added to the sufficient ration.

Eggs from both groups were set five weeks after the pullets had been put onto their respective rations. A second hatch was set two weeks later and subsequently eggs were set every week.

After the first two hatching results the pullets were classified on the basis of fertility and hatchability of their eggs. Five pullets were classed as culls in each group. The culls were maintained in their respective groups but were not used in recording production and hatchability data. With regard to vitamin A reserves, it was considered that they would be representative of the group as their egg production and bodyweight were comparable with those of other pullets in the group. They were killed at intervals to determine vitamin A reserves, but with one exception in D Group, not until after the reversal of the trial.

At intervals before the reversal of the experiment, the two poorest producers in S Group and the four poorest producers in D Group were killed to determine liver vitamin A reserves. In addition, one cull from D Group was killed for the same purpose. The pullets in D Group were examined approximately twice a week for field evidence of vitamin A deficiency. The incidence of "pustules" in the oesophagus (henceforth referred to as nodules) was taken as field evidence of vitamin A deficiency.

The experiment was reversed after nodules on the oesophagus were detected in all pullets in D Group by field examination. S Group was divided into two equal groups of 14 pullets on the basis of their weights and prior egg production, and these are designated the SS and SD Groups. SS Group was maintained on the sufficient ration and SD Group was placed on the deficient ration. D Group was divided into two unequal groups—a DD Group of 10 pullets maintained on the deficient ration and a DS Group of 14 pullets placed on the sufficient ration. Thus SS and DS Groups were in one pen, and DD and SD Groups in the other. These four groups were treated separately in recording production and hatchability data.

This subdivision into four groups was an opportunity for the further culling of pullets from S and D Groups known to be abnormal. One additional cull was selected from each group. This made six culls from S Group, of which two were maintained in SS Group and four placed in SD Group. The four culls from D Group were all placed in DS Group. These culls were killed at intervals to assess liver vitamin A reserves. In addition, day-old chickens from SS, SD and DS Groups were analysed for total liver vitamin A reserves at hatching. Chickens from DD Group were not available, the hatchability in this group being nil at this stage.

All the pullets in Groups SS and DS were killed a week after the occurrence of nodules on the oesophagus in three pullets from SD Group. They were examined for parasite burden and their livers analysed for vitamin A. Survivors of Groups DD and SD were maintained on the deficient ration until the trial terminated on May 20, 1957.

(3) Chemical Analysis.

A chromatographic method was sought that could be used to separate vitamin A from the carotenoids present in yolks and in yolk-sacs and that at the same time could be used for the determination of vitamin A added to feeds. A method based on the "Report on Vitamin A in Mixed Feeds" (Cooley 1952) was tried. It was found to be unsuitable for our particular requirements and was modified as follows:

(a) Vitamin A in Yolks and Yolk-Sacs.

For each analysis the yolks from six hard-boiled eggs were mixed and 12 g. taken for saponification. Composite yolk-sac samples from four chickens were taken for saponification. The saponification and extraction procedure was that used for butterfat by McDowell (1949).

An aliquot of the sulphuric ether extract, normally 20 ml., was evaporated to dryness on a water bath in a 150 ml. Erlenmeyer flask. Two drops of pyridine and 0·5 ml. acetic acid anhydride were added and the acetylation carried out on a water bath. At all stages of evaporation and acetylation a gentle stream of carbon dioxide was directed over the solution. When most of the acetic acid anhydride had been driven off, a further 0·5 ml. was added together with two drops of pyridine and the acetylation continued. Most of the excess acetic acid anhydride, pyridine and by-products of acetylation were driven off with carbon dioxide. To render the product totally free from these polar substances, two lots of about 25 ml. petroleum ether (Shell X222, fraction boiling between 60 deg. and 80 deg. C.) were added after the second acetylation and evaporated off. The vitamin A was now in the form of vitamin A acetate and was taken up in 50 ml. petroleum ether. Acetylation in dilute solutions, or acetylation using only one lot of acetic acid anhydride and pyridine, gave incomplete conversion of vitamin A alcohol to vitamin A acetate.

The activation of the magnesia (Microbrand No. 2641) was the same as described by Cooley (1952). The chromatographic tube consisted of a reservoir 40 mm. in diameter and 100 mm. long sealed to tubing 18 mm. in inside diameter and 250 mm. long to which a capillary tube 75 mm. long with about a 1 mm. bore was sealed. The height of the magnesia plus hyflo-super-cel was 85 mm. and a further 10 mm. layer of anhydrous sodium sulphate was added. The column was washed with 50 ml. petroleum ether immediately prior to use. The 50 ml. acetylated extract was then passed through the column, and as soon as the liquid layer reached the top of the adsorbent a further 20 ml. petroleum ether was added to displace the remainder of the acetylated extract from the column.

Depending on the amount of vitamin A present in the original sample, either all the eluate and washings or an aliquot was taken for the Carr-Price reaction. A correction for 20 per cent. loss had to be made in this method.

About half of this was due to acetylation losses. The other half was due either to vitamin A adsorbed on the column in the form of the alcohol, through incomplete acetylation, or to destruction of vitamin A in the process of analysis.

For yolks containing normal amounts of vitamin A, the acetylation and chromatographic steps may be omitted without loss of accuracy. However, when the yolk contains little or no vitamin A, these steps are necessary to avoid interfering purple or green colours in the Carr-Price reaction due to the presence of carotenoids, mainly lutein. Presumably the blue colour due to vitamin A is developed before the other interfering colours. Chromatographed extracts of vitamin A deficient yolks or yolk-sacs still gave a slight galvanometer deflection with the Carr-Price reagent even though the solution remained colourless. For these samples, 1 I.U. was subtracted from the readings.

(b) Vitamin A in Feeds.

Saponification followed by extraction with petroleum ether was used for feed analyses in preference to a direct solvent extraction. Stabilized vitamin A added to poultry rations sometimes contains a protective coating insoluble in petroleum ether. Also, with a direct solvent extraction fat interferes with the Carr-Price reaction, particularly in samples low in vitamin A.

The saponification was carried out by refluxing 20 g. of feed for 10 min. with 5 ml. saturated potassium hydroxide solution and 50 ml. ethyl alcohol. The material was centrifuged and 25 ml. of the supernatant liquid extracted with 45 ml. petroleum ether and 20 ml. water. This was followed by a second extraction with 45 ml. petroleum ether. The extracts were combined and washed twice with about 100 ml. water. They were then made up to 100 ml. with petroleum ether and dried over anhydrous sodium sulphate. The solution was then acetylated and chromatographed as described above. Normally 50 ml. was taken for acetylation and after chromatography the eluate was made up to 50 ml., of which 25 ml. was taken for analysis. As in yolk analysis, 1 I.U. was subtracted from the readings in samples containing little or no vitamin A as seen by the absence of any blue colour, although small galvanometer deflections were still recorded.

III. RESULTS OF 1955 PILOT TRIAL.

(a) Egg Production.

There were no group differences in egg production throughout the trial.

(b) Hatchability.

The hatchability was calculated from the total number of chickens hatched and the total number of eggs set.

Eggs from both groups were set four weeks after the commencement of the trial, then at weekly intervals for 21 consecutive weeks. The hatchability fluctuated in the group on the sufficient ration from 78·79 per cent. to 45·16 per cent. and in the group on the deficient ration from 77·33 per cent. to 40·74 per cent. There was no regular pattern in the results, except that the group on the deficient ration tended to produce eggs of lower hatchability than the control group. This was neither constant nor significant and was due to a higher percentage of infertile eggs in this group (average 17·1 per cent., against 8·9 per cent. in the group on the sufficient ration). Artificial insemination of the hens from both groups in the last four hatches using Australorp males did not reduce the number of infertile eggs. Presumably some hens in both groups were sterile. It was decided to adopt trap-nesting in further work to enable infertile and unproductive hens to be identified at the commencement of the experiment.

(c) Liver Vitamin A Reserves of Hens.

The hens in this experiment had been used previously in a Random Sample Trial. The number was too small to permit any to be slaughtered to determine initial vitamin A reserves. A sample of the ration fed for the previous 18 months was analysed; it contained 12,300 I.U. of vitamin A per lb. This was over four times the amount of vitamin A added to the ration. An analysis of the liver meal used as a component of the ration (3½ per cent.) proved it was responsible for the additional vitamin A. The year the liver meal was produced and the year before that were periods of abundant rainfall in Queensland. This would result in pastures of high carotene content throughout the year and must have produced good reserves of vitamin A in the livers of grazing animals. In spite of processing, the liver meal was thus rich in vitamin A.

Table 1.

Liver Vitamin A Reserves of Hens on Vitamin A Sufficient and Deficient Rations (1955).

Weeks From Commencement of Trial.	Liver Vitamin A (μg./g.).		Remarks.
	Sufficient Ration.	Deficient Ration.	
5	840	..	Killed
10	..	780	Died
11	828	..	Killed
11½	711	..	Died
20	..	400	Died
31	..	108	Died
33 (end of trial) ..	1,060	1,171	Killed
	822	566	Killed
	655	541	Killed
	477	479	Killed
	270	..	Killed

It was anticipated that the initial liver vitamin A reserves of these hens would be high, but depletion in three to six months was expected in the group on the deficient ration. Analytical data are presented in Table 1. The liver reserves were high in both groups when the trial was terminated after 33 weeks.

(d) Vitamin A Content of Egg Yolk.

As shown in Table 2, both groups produced eggs containing comparable amounts of vitamin A throughout the experiment. The eggs did not contain many pigments, for the ration was low in carotenoids. The carotenoid content ranged from 100 μg. per egg yolk at the beginning of the trial to less than 20 μg. at the end, of which about 7 per cent. was beta-carotene. Sjollema and Donath (1940) found that most of the carotenoid pigments in the yolk are in the form of xanthophylls, the usual ratio of carotenes to xanthophylls being 1:10.

Table 2.

VITAMIN A CONTENT OF YOLKS FROM HENS ON VITAMIN A SUFFICIENT AND DEFICIENT RATIONS (1955).

Weeks From Commencement of Trial.	Vitamin A in Yolks (μg.).	
	Sufficient Ration.	Deficient Ration.
6	118	112
11½	121	103
21½	72	85
32½	107	101

(e) Vitamin A Reserves of Chickens at Hatching.

Newly hatched chickens were analysed for vitamin A reserves on three occasions during the experiment. There were no differences between groups. The total vitamin A reserves at hatching averaged 34 μg., of which 12 μg. was in the liver and 22 μg. in the yolk-sac. Some of these chickens were hatched from eggs, the vitamin A contents of which were known by previous analyses of composite egg yolk samples. The average vitamin A reserves of newly hatched chickens was only one-third of the content of the unhatched eggs.

The distribution of vitamin A between yolk-sac and liver was examined in chickens from two hatches which were not given access to food. The experimental procedure involved slaughter of four chickens and the analysis of composite samples of yolk-sacs and livers. In one case chickens were killed two, three and four days after hatching. On the next occasion chickens were killed at hatching, one day later and two days later. There was no regular pattern in their total vitamin A reserves, but at hatching 65 per cent. of

the total vitamin A reserves was in the yolk-sac; the reserves dropped to 63, 48, 37 and 33 per cent. in successive days. Parrish, Williams, Hughes and Payne (1950) found that 60 per cent. of the vitamin A in the chicken at hatching was in the residual yolk.

IV. RESULTS OF 1956 TRIAL.

(1) Effects of Vitamin A Deficiency in Pullets.

(a) Egg Production.

Eggs laid by 31 pullets from each of Groups S and D were considered in calculating the number of eggs laid per pullet per week. The eggs laid by pullets noted as culls for infertility and poor hatchability were excluded.

At the end of the first stage of the trial, prior to separation into four groups, there were 29 pullets left in S Group and 25 in D Group, both excluding culls. At the end of the second stage of the trial, one week after the incidence of nodules on the oesophagus in SD Group, there were 14 pullets in SS Group, 13 in Groups DS and SD and five in DD Group.

The egg production over the whole period of the trial is illustrated in Fig. 1. The first stage comprised 18 weeks. For the first three weeks the egg production was higher in D Group. Thereafter it declined gradually, until at the reversal point, when field evidence of vitamin A deficiency was apparent in this group, the pullets were laying 1·38 eggs per week, compared with 4·66 eggs per week in S Group. The average weekly egg production in D Group over the whole period of 18 weeks was 3·46, compared with 4·69 in S Group. These differences, though significant, would have been greater if three of the poorest layers had not been in S Group and if four poor layers had not been taken from D Group for analysis against only two from S Group.

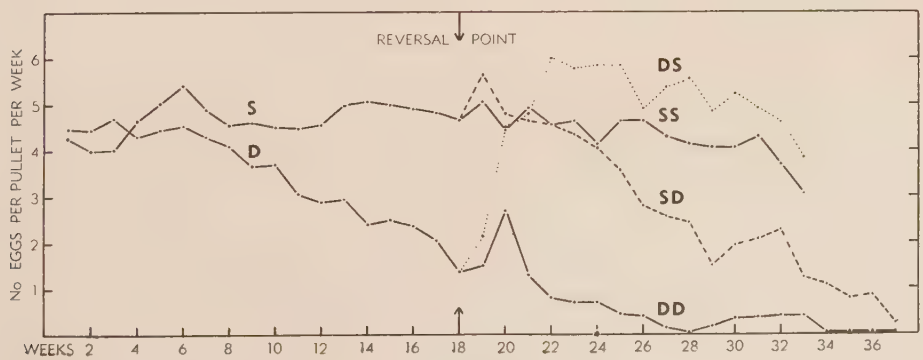

Fig. 1.

Egg Production Over the Period of the Trial.

The post-reversal stage of the trial was the period of 14 weeks from the 18th to the 32nd week. SS Group maintained an even egg production until just before they were killed, when hot weather caused a general drop in egg production. The egg production of DS Group was back to normal only two weeks after supplementation with vitamin A and thereafter was consistently higher than in SS Group. SD Group followed the same pattern as D Group, and when nodules were evident in this group at the 32nd week, the pullets were laying 2·28 eggs per week, against 3·71 in SS Group and 4·62 in DS Group.

From the individual egg production records, it was evident that in the depleted D and SD Groups the cessation of egg production was gradual in most instances. This is contrary to the findings of Taylor *et al.* (1947), who found on examination of individual records that death occurred about 12 days after the last egg was laid and that very little drop in production occurred prior to that time. McClymont and Hart (1948) reported that the rate of egg production was only slightly affected before cessation of production.

There was a slight and temporary increase in egg production in DD Group at the second week after reversal. The possibility that pullets in this group may have ingested small amounts of vitamin A from the faeces of SD Group, now in the same pen, was examined. Chemical analysis of faeces failed to show a measurable amount of vitamin A.

(b) Hatchability.

The number of pullets in the different groups from which eggs were set was the same as recorded for egg production. For the first hatch 135 eggs were set from both S and D Groups and equal numbers of eggs from each group were set for the next five hatches. For the last six hatches prior to reversing the groups, more eggs were set from S Group than from D Group. This was due to a marked fall in egg production in D group and a high requirement of chickens for other purposes. The last three hatchability values of D group resulted from 45, 52 and 27 eggs set. After reversing the groups, all suitable eggs laid were set.

The percentage hatchability over the whole period is illustrated in Fig. 2. For the 18 weeks' period prior to reversal, the hatchability of S Group maintained a normal level, while that of D Group dropped sharply. Individual hatchability and production data are not presented but examination of these records showed:

(1) In S Group two pullets laid eggs of much lower hatchability (58·14 and 37·50 per cent.) than the average for the group.

(2) In D Group, the eggs laid by two pullets accounted for 60 per cent. of the chickens hatched in the last five hatches. One of these pullets was the second highest producer in its group and the hatchability of its eggs

(average 85·19 per cent.) was only beginning to drop in the last batch of eggs set. Presumably these two pullets had greater vitamin A reserves at the commencement due to more efficient utilization.

(3) Vitamin A deficiency affects the percentage hatchability of eggs from individual pullets before it affects their egg production.

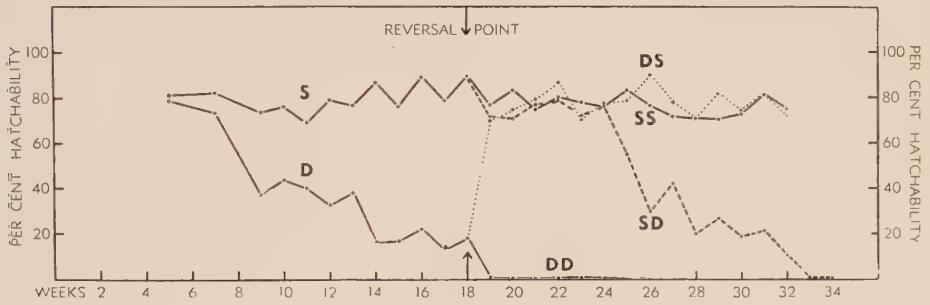

Fig. 2.

Hatchability Over the Period of the Trial.

Table 3 presents data on the time interval between a drop in hatchability and a drop in egg production from 20 pullets in D Group.

Table 3.

Weeks Intervening Between Drop in Hatchability and Drop in Egg Production from 20 Pullets in D Group.

Intervening Period (weeks)	−1.	0.	+2.	+3.	+4.	+5.	+7.
No. of Pullets ..	1	1	4	4	8*	1	1

*One pullet ceased egg production four weeks before the last hatch; in the last week it laid three eggs, from which three chickens hatched.

Table 4.

Stage of Incubation at Which Mortality Occurred in Groups S and D.

Group.	Infertile Eggs.	Dead Embryos at :			Pipped.	Failed to Hatch.	Hatchability.
		5 days.	13 days.	18 days.			
	%	%	%	%	%	%	%
S	3·9	2·3	3·4	1·4	2·3	6·2	80·5
D	4·2	9·5	11·0	6·5	4·5	21·1	43·2

In Table 4 the stage of incubation at which mortality occurred in S and D Groups is presented.

The hatchability in DS Group was back to normal after only one week's access to a ration containing normal amounts of vitamin A. The hatchability in SS Group was comparable with that recorded for S Group. The hatchability in SD Group decreased in much the same way as the hatchability in D Group and was down to nil one week after the incidence of nodules on the oesophagus. The hatchability in DD Group was nil one week after the incidence of nodules on the oesophagus in D Group—i.e., one week after the reversal point—and remained at this level until egg production virtually ceased. The maximum number of eggs set in DD Group to give zero hatchability was 18, against 16 in SD Group.

Examination of individual records showed:—

(1) Two pullets in SS Group laid eggs of much lower hatchability (41·03 and 12·82 per cent.) than the average for the group. The one with the lowest percentage hatchability was also the lowest when it was in S Group. This was due to the production of infertile eggs. In DS Group two pullets also laid eggs of appreciably lower hatchability (51·28 and 47·54 per cent.) than the average for the group.

(2) In SD group the eggs laid by two pullets accounted for 65·8 per cent. of the chickens hatched in the last six hatches, one of these pullets being a good layer and the best producer in its group.

(3) Vitamin A deficiency affects the percentage hatchability of eggs from individual pullets before it affects their egg production. This is summarised for 11 pullets in Table 5.

Table 5.

Weeks Intervening Between Drop in Hatchability and Drop in Egg Production from 11 Pullets in SD Group.

Intervening Period (weeks)	+1.	+2.	+4.	+6.
No. of Pullets ..	5	3	2	1

The stage of incubation at which mortality occurred in SS, SD, DS and DD Groups is shown in Table 6

Table 6.

Stage of Incubation at Which Mortality Occurred in Groups SS, SD, DS and DD.

Group.	Infertile Eggs.	Dead Embryos at:			Pipped.	Failed to Hatch.	Hatchability.
		5 days.	13 days.	18 days.			
	%	%	%	%	%	%	%
SS	4·0	3·7	9·2	1·8	0·8	3·6	76·9
SD	2·5	8·4	11·1	4·7	3·0	14·0	56·3
DS	2·6	4·1	6·5	2·5	0·5	5·5	78·3
DD	4·1	21·9	48·0	17·8	0·0	8·2	0·0

From Tables 4 and 6 it is evident that although vitamin A deficiency in the pullet affects the hatchability of eggs, it had no effect on the fertility. The range of infertile eggs for individual hatches in all groups was 0–9·8 per cent.

During the course of the trial 4,607 eggs were set and an overall fertility of 96·5 per cent. resulted. In a comparative test of artificial insemination *versus* natural mating over a hatching period of 300 days, Cooper (1955) obtained an overall fertility of 83·4 per cent. in his inseminated pens.

(c) Liver Vitamin A Reserves of Pullets.

Prior to separating the pullets into their respective groups at the commencement of the trial, four were killed to determine their liver vitamin A reserves. They were all normal (range 239–156 μg./g.). The liver vitamin A

Table 7.

LIVER VITAMIN A RESERVES OF PULLETS IN RELATION TO TIME FROM COMMENCEMENT OF TRIAL

Weeks From Commencement of Trial.	Liver Vitamin A (μg./g.)					Remarks.
	S.	D.	SS.	SD.	DS.	
1½	76	113	..	..	..	Killed
8½	220	6	..	..	..	Killed
9	..	2·5	..	..	..	Killed
10	132	1	..	..	..	Killed
11	..	<1	..	..	..	Killed (cull)
11½	..	<1	..	..	..	Killed
16	..	<1	..	..	..	Died
18	..	<1	..	..	..	Died
		REVERSAL.				
20½			..	..	4	Died
21			..	115	..	Killed (cull)
25½			26	<1	25	Killed (cull)
25½			..	<1	..	Killed (cull)
30			..	<1	34	Killed (cull)
30			..	..	9	Killed (cull)
31½			69	..	..	Killed (cull)
33½ (end of trial)			151	..	71	Killed
			143	..	64	Killed
			142	..	51	Killed
			140	..	47	Killed
			94	..	40	Killed
			91	..	36	Killed
			82	..	35	Killed
			75	..	35	Killed
			53	..	32	Killed
			46	..	23	Killed
			43	..	20	Killed
			35	..	18	Killed
			32	..	8	Killed
			15	..	4	Killed

reserves of all the pullets killed and of some that died during the experiment, and the time interval from commencement of the trial, are set out in Table 7. The egg production and hatchability of these periods, shown in Figs. 1 and 2, can thus be related to the vitamin A reserves in the pullets. The liver vitamin A levels were interpreted in the manner discussed by Gartner and Ryley (1956), in which levels between 0 and 2 μg./g. were considered indicative of vitamin A deficiency and from 2+ to 10 μg./g. were considered marginal.

S Group.—As would be expected, the pullets in this group had normal liver vitamin A reserves.

D Group.—The liver vitamin A reserve of a pullet was marginal 8½ weeks after commencement of the trial. At this stage the egg production of the group had been falling gradually and was less than the egg production of S Group. At the same stage there was an even greater difference in hatchability, D Group's being of the order of less than 50 per cent. Pullets killed subsequently showed decreasing reserves until they reached levels indicative of vitamin A deficiency.

SS Group.—The culls that were killed had normal reserves of vitamin A. At the end of the trial all the pullets were killed to determine the level and distribution of vitamin A in the group. The average level (82 μg./g.) was the same as found by Gartner and Ryley (1956) in their survey of commercial flocks in Queensland.

SD Group.—The liver vitamin A level of a pullet in this group was deficient 7½ weeks after reversal. As in D Group, egg production and hatchability had declined at this stage.

DS Group.—A pullet that died 2½ weeks after reversal had a marginal liver vitamin A level. The liver reserves were normal in another pullet 7½ weeks after reversal. The average reserve at the end of the trial was 35 μg./g.; two pullets had marginal reserves; the rest were normal.

DD Group.—Livers from pullets that died in this group were not analysed for vitamin A, as extremely low reserves of vitamin A in D Group had been established prior to reversal.

(d) Vitamin A Content of Egg Yolks.

Six eggs were selected at random from each group and the yolks bulked for analysis. When vitamin A deficiency was first confirmed in D Group by liver analysis, the eggs laid by this group contained less than 8 μg. of vitamin A per yolk, against 42 μg. per yolk in S Group. Two further analyses, each at fortnightly intervals, revealed that the vitamin A content of eggs from D Group decreased still further until the exact amount present, if any, was indeterminable by the chemical method employed. The vitamin A content of eggs from S Group was 62 and 48 μg. per egg over the same period. These levels in S Group were lower than those obtained in eggs during the 1955 trial (121–72 μg./yolk). No eggs were analysed after reversal.

(e) Vitamin A Reserves of Chickens at Hatching.

The vitamin A reserve in day-old chickens from SS, SD and DS Groups was determined (Table 8). Each analysis represents composite liver and yolk-sac samples from four chickens.

Table 8.

VITAMIN A RESERVES IN THE LIVER AND YOLK-SAC OF CHICKENS AT HATCHING.

Weeks After Reversal.	Liver Vitamin A (μg.).			Yolk-sac Vitamin A (μg.).			Total Vitamin A (μg.).		
	SS.	SD.	DS.	SS.	SD.	DS.	SS.	SD.	DS.
2	13	22	13	11	16	11	24	38	24
3	20	8	12	10	3	4	30	11	16
4	19	6	14	12	2	8	31	8	22

Reference to the hatchability data (Fig. 2) shows that the period, four weeks after reversal, was three weeks before the hatchability in SD Group began to fall. At this stage the total vitamin A reserve in the SD chickens was 8 μg., compared with 11 and 38 μg. in the preceding weeks. It is apparent that there was a marked fall in the vitamin A reserves of chickens at hatching prior to the effect of a vitamin A deficient ration on the hatchability of eggs.

(f) Field Examination for Lesions of Vitamin A Deficiency.

Depleted pullets were examined approximately twice a week for nodules on the oesophagus. This was done prior to artificial insemination and thus did not entail extra handling. Feed was removed at least two hours before examination. This was desirable, as in our experience food particles can be confused with small nodules.

In D Group nodules were first detected in four pullets and three days later in the remainder. In SD Group there was a lag of 11 days from the time nodules were detected in three pullets until the last two in the group exhibited lesions.

The disappearance of nodules after vitamin A supplementation may be a slow process. In DS Group, although nodules could not be detected in three pullets at four days after supplementation, it took eight weeks for nodules to disappear in the last three pullets.

(g) Principal Autopsy Findings.

Table 9 sets out the principal autopsy findings of all the pullets that died during the trial. After field evidence of vitamin A deficiency had been established, nodules on the oesophagus were found in eight of the 10 pullets that died in DD Group and in eight of the 10 pullets that died in SD Group. The pullets not exhibiting these lesions were all in an advanced stage of decomposition when autopsied. Yet by field examination nodules were detected in them, at the most, a week prior to death.

Table 9.

PRINCIPAL AUTOPSY FINDINGS OF PULLETS THAT DIED DURING THE TRIAL.

Group.	Weeks Deficient as Determined by		Principal Autopsy Findings.
	Chemical Evidence.	Field Evidence.	
D	2	..	Died during treatment with carbon tetrachloride ; kidneys friable, suggestive of cloudy swelling ; liver extremely friable
D	6	..	Myocardial degeneration ; kidneys extensively mottled ; liver swollen and soft
D	7	..	Large haematoma of right lobe of liver ; liver enlarged, very friable and soft ; kidneys enlarged, mottled
D	8	0	Liver extremely friable with sub-capsular haemorrhage ; 12 early nodules on oesophagus ; kidneys cloudy swelling
DS	..	..	Peritonitis due to abrasion of cloaca when being artificially inseminated ; few nodules left on oesophagus
DD	14½	6½	Egg peritonitis ; nodules on oesophagus ; kidneys swollen, suggestive of cloudy swelling ; liver friable
DD	16	8	Nodules on oesophagus ; kidneys pale, with slight frosting
DD	18½	10½	Egg peritonitis ; nodules on oesophagus ; kidneys swollen
DD	19	11	Nodules on oesophagus ; kidneys swollen, uniformly frosted; ureters distended with urates
DD	21	13	Massive sub-capsular haemorrhage of liver ; no nodules on oesophagus ; advanced stage of decomposition
SD	6	..	Egg peritonitis ; kidneys coarse mottling ; ureters slightly distended with urates ; fatty degeneration of liver
DD	25	17	No nodules on oesophagus ; advanced stage of decomposition
SD	13½	6½	Large blood clot on both sides of liver ; liver friable ; pale kidneys ; no nodules on oesophagus ; decomposed
SD	15	8	Visceral gout ; deposit of urates on kidneys, epicardium and pericardium ; ureters distended with urates ; no nodules on oesophagus ; decomposed
SD	15½	8½	Visceral gout ; nodules on oesophagus ; kidneys pale ; thick deposit of urates over liver, heart ; ureters distended with urates
SD	16	9	Nodules (difficult to detect) on oesophagus ; kidneys white ; urates on epicardium ; ureters distended with urates ; decomposed
DD	31	23	Visceral gout ; nodules on oesophagus ; deposit of urates on kidneys, liver, epicardium and intestines ; ureters distended with urates
DD	32½	24½	Nodules on oesophagus ; kidneys frosted ; ureters distended with urates
SD	17½	10½	Visceral gout ; nodules on oesophagus ; kidneys white ; thick layer of urates on kidneys, liver, pericardium and epicardium
SD	17½	10½	Nodules on oesophagus ; liver enlarged, with slight, patchy haemorrhages
SD	17½	10½	Nodules on oesophagus
SD	18½	11½	Few nodules (on pharynx only) ; decomposed
SD	19	12	Nodules (on pharynx only) ; urates in ureters and in kidneys ; decomposed.
SD	21	14	Visceral gout ; few nodules (difficult to detect) on oesophagus ; kidneys markedly pale ; urates in kidneys and ureters ; deposit of urates on liver and lung ; decomposed
DD	37½	29½	Mouth to crop heavily studded with nodules ; kidneys pale, swollen and enlarged ; ureters distended with urates
DD	39½	31½	Nodules on oesophagus, pharynx, tongue ; kidneys pale, with deposition of urates ; ureters grossly distended with urates

Of the 24 deaths in the deficient groups (excluding the one that died after treatment with carbon tetrachloride), 10 were due to uncomplicated vitamin A deficiency and five to visceral gout. Hungerford (1951) stated that visceral gout is a condition seen as a symptom of ureamia and in acute vitamin A deficiency. Four deaths were due to either a partial rupture of the liver with sub-capsular haemorrhage or a complete rupture of the liver with intra-abdominal haemorrhage, three to egg peritonitis, one to myocardial degeneration and one pullet was too decomposed to enable a diagnosis. It must be remembered that vitamin A deficiency was probably a contributing factor in all deaths and certainly was the only cause of death in 15 pullets.

In addition, an autopsy was done on most of the pullets killed for liver vitamin A assay. In SD Group two pullets showed fatty degeneration of the liver and in another pullet from the same group the abdomen contained fluid from a ruptured yolk.

(h) Field Observations.

Eggs laid by the five culls in both S and D Groups were lighter in colour throughout the trial than the eggs laid by the other pullets. Shell texture of all eggs was good throughout, even in the vitamin A deficient groups.

There was a marked difference in appearance between the chickens of pullets on sufficient and deficient rations after hatchability in the deficient groups had started to decline. The chickens from the sufficient groups dried out well and were first-class by the 22nd day, whereas the chickens from the deficient groups were still sticky and on appearance would be culled in accordance with commercial hatchery practice.

The feeding habits of both groups were similar. When pullets became vitamin A deficient, they frequented their nests just as often, even after laying had virtually ceased.

Apart from the incidence of nodules on the oesophagus, a casual inspection of the respective groups would not enable the deficient pullets to be singled out. Closer observation revealed that vitamin A deficient pullets became listless after their egg production dropped. They did not work the litter and their droppings, accumulating under the perches, had to be cleared out weekly and the litter stirred. The vitamin A sufficient pullets worked their litter as normal, healthy fowls do.

None of the pullets that succumbed to vitamin A deficiency was emaciated. In fact, the longest lived survivor from DD Group was still in good condition when it died 39½ weeks after vitamin A deficiency had been established in this group.

(i) Food Consumption.

Food consumption was recorded in S and D Groups over the last 39 days of the first part of the trial after D Group had been found to be vitamin A deficient by chemical analysis. Over this period S Group consumed 5·13 oz. of feed per pullet per day, against 4·89 oz. by D Group. This difference of 0·24 oz. per fowl per day is not considered significent. No records were kept after reversal, since there were then two groups in each pen.

(j) Weights of Pullets.

The pullets were individually weighed at the commencement of the trial, once prior to reversal, at reversal, and then at fortnightly intervals. The last weighing was when all survivors in SS and DS Groups were killed. There was no difference in the average weight gains (Table 10) between S and D Groups. The weights in SD Group were comparable with those in SS Group up to the time nodules were detected in the former group, but a week later the average weight in SD Group dropped by 5 oz. The heaviest pullet was in DD Group; it weighed 9 lb. 8 oz. at death. However, the average weight of the five survivors was considerably less than in DS Group.

Table 10.

AVERAGE WEIGHT OF PULLETS FROM EACH GROUP AT CERTAIN PERIODS OF THE TRIAL

Period.	S.	D.	SS.	SD.	DS.	DD.
	Lb. oz.	Lb. oz.	Lb. oz.	Lb. oz.	Lb. oz.	Lb. oz.
Commencement of Trial	5 3½	5 5½				
At Reversal	6 1	6 3				
Difference	+ 13½	+ 13½				
Commencement of Reversal			6 1	6 1	6 4	6 2
Week After Nodules in SD Group ..			6 5	5 13*	6 13*	5 9†
Difference			+ 4	− 4	+ 9	− 9

*13 survivors out of 14.
†5 survivors out of 14.

(k) Ascaridia galli Infestation.

In the first part of the trial, there were indications that a heavier infestation of *Ascaridia galli* was present in D Group than in S Group. Nine weeks before reversal a pullet killed in D Group had a few mature worms. The pullets in both groups were then dosed with 1 ml. of carbon tetrachloride to control the parasites. Two weeks after treatment another pullet killed in D Group had 20 adult *Ascaridia galli*. The treatment with carbon tetrachloride was repeated, this time after a starvation period of 12 hours. One of the pullets in D Group, which died during treatment, harboured 85 worms, of which 10 per cent. were fourth-stage. About six hours after treatment a

composite faecal sample collected from 12 pullets in each group revealed 30–60 eggs per gram of faeces in S Group and 300–400 e.p.g. in D Group. A week later a further egg count showed no eggs in S Group and 100 e.p.g. in D Group

Thereafter a proprietary piperazine compound was used to avoid the danger of liver damage from repetitive and frequent dosing with carbon tetrachloride. Five weeks before reversal the pullets were starved overnight and the anthelmintic incorporated in the ration for one day. The treatment apparently removed all the adult worms, the egg counts being negative, but $1\frac{1}{2}$ weeks later 100 e.p.g. were again present in a composite sample from D Group. A further treatment eradicated the infestation and no eggs were voided by either group until three weeks after reversal—i.e., for $8\frac{1}{2}$ weeks.

After reversal, SS and DS Groups were in one pen and SD and DD Groups in another. For this stage of the trial, correlation of *Ascaridia galli* infestation with the vitamin A status was less satisfactory. From the 5th to the 10th week after reversal egg counts were done at weekly intervals on bulked faeces. With one exception, SS and DS Groups showed fewer eggs per gram than SD and DD Groups. At the 15th week after reversal all the pullets in SS and DS Groups, having been killed, were examined for *Ascaridia galli*. The average number of worms was 1·9 in pullets from SS Group and 9·6 in pullets from DS Group. At the same time SD and DD Groups were treated with a piperazine compound and a count made of all the worms voided. The average figures were 3·6 worms in pullets from SD Group and 5·0 worms in pullets from DD Group. These are not considered pathogenic burdens. Individual variation within groups makes these results not statistically significant.

(1) Survival Time after Incidence of Vitamin A Deficiency.

The survival time after the incidence of vitamin A deficiency as diagnosed by chemical analysis is shown for DD and SD Groups in Fig. 3. In DD Group the range was $14\frac{1}{2}$ to $39\frac{1}{2}$ weeks with a mean survival time of $25{\cdot}50 \pm 9{\cdot}09$ weeks. In SD Group the range was 6 to 32+ weeks, there being one survivor when the trial was terminated. Taylor *et al.* (1947) found that in one experiment, at a feeding level of 540 I.U. of vitamin A per lb. of feed, over 80 per cent. of the pullets died within six months of the time vitamin A deficiency was first observed. McClymont and Hart (1948) found that the time between cessation of egg production and death from vitamin A deficiency was $44{\cdot}7 \pm 17{\cdot}3$ days. In DD Group the time between cessation of egg production and death from vitamin A deficiency was $64{\cdot}0 \pm 38{\cdot}4$ days.

The mean weekly maximum temperature and the mean weekly relative humidity are shown graphically in Fig. 3. For any particular week, the mean values for temperature and humidity are recorded for the preceding week, since if these factors affect mortality rate, it would be due to their influence in the days preceding death.

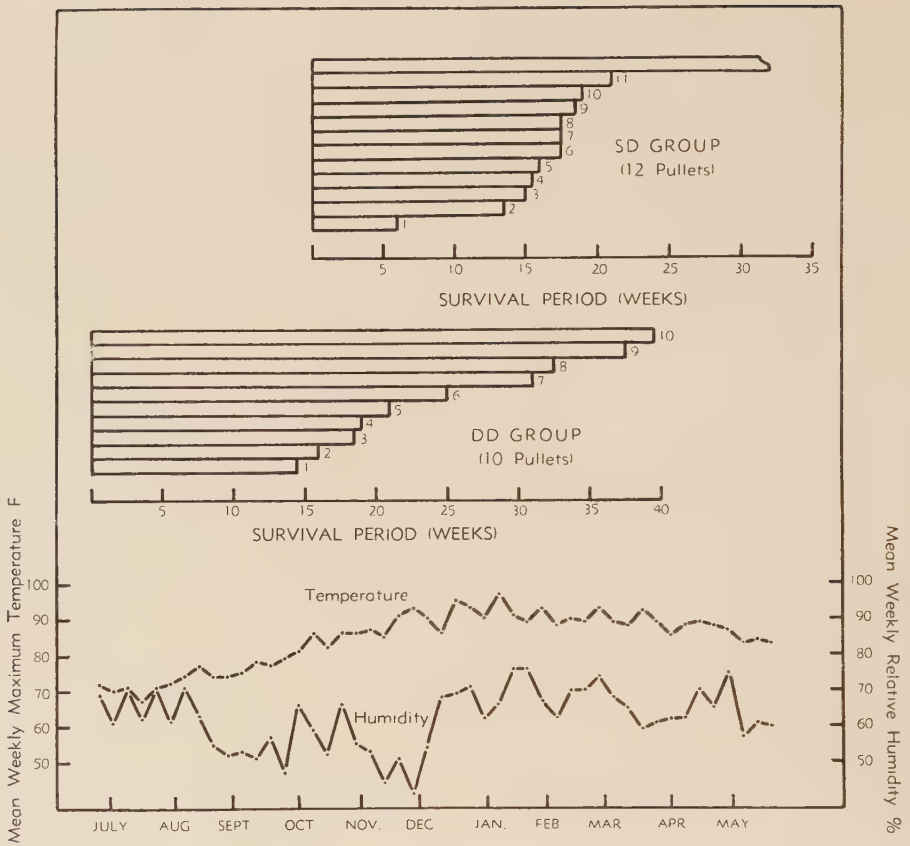

Fig. 3.

Survival Following Chemical Diagnosis of Vitamin A Deficiency in Groups DD and SD, together with Temperature and Humidity Records. The numbers alongside the histograms represent total number of deaths. Since the submission of this paper the 12th pullet from SD group died at 35 weeks.

As seen in Fig. 3, both temperature and humidity were higher in the initial period of deficiency in SD Group than in the initial period of deficiency in DD Group. There were only 5½ weeks intervening between the second and 10th deaths in SD Group. High temperatures and high humidity may have furnished additional stress, thereby contributing to the mortality. In DD Group there were only four survivors at the beginning of this period and two of them died before its end.

(2) Effects of Vitamin A Deficiency in Chickens.

(a) General.

Experimental facilities limited the amount of investigational work on chickens. Chickens were reared up to four weeks of age from four consecutive hatches of eggs from S and D Groups. The first lot was hatched from eggs

laid 10 weeks after commencement of the trial, when egg production and hatchability of D Group had fallen and there was chemical evidence of vitamin A deficiency. The eggs laid by D Group at the time were low in vitamin A and the few day-old chickens analysed had no liver vitamin A reserves. Thus the day-old chickens from D Group were considered vitamin A deficient, while those from S Group were considered vitamin A sufficient. The day-old chickens were divided into four groups:—

ds Group: deficient chickens on a vitamin A sufficient ration.

dd Group: deficient chickens on a vitamin A deficient ration.

ss Group: sufficient chickens on a vitamin A sufficient ration.

sd Group: sufficient chickens on a vitamin A deficient ration.

The ds Group was omitted from the last two rearing experiments. Instead, a dss Group was included in which deficient chickens were reared on a ration containing five times (14,000 I.U./lb.) the amount of vitamin A usually used.

Because the chickens were to be reared for only one month, the chickens were fed, instead of a starter ration, the same ration as used for the adult fowls. The calcium intake was thus lower than normal. This may have been responsible for a disorder, resembling rickets, in some chickens in sd and dd Groups at the first rearing trial. However, chickens in the subsequent rearing trials did not show this disorder.

There was the same number of chickens in the different groups in each of four rearing experiments, these being 14, 13, 12 and 12 respectively. This number was limited in each instance to the number of chickens hatched from D Group. For convenience, the results are presented as representative of 51 chickens in dd, ss and sd Groups and of 27 chickens in ds and dss Groups.

(b) Rearing Mortality.

The rearing mortality up to four weeks was:—

dd Group: 71 per cent.
ds Group: 41 per cent.
dss Group: 17 per cent.
sd Group: 25 per cent.
ss Group: 4 per cent.

The heaviest mortality occurred in ds Group 4–6 days after hatching, in dd Group 3–7 days after hatching, and in sd Group 5–6 days after hatching.

(c) Liver Vitamin A Reserves of Chickens.

Three chickens from each group were analysed individually at four weeks of age. As shown in Table 11, the liver vitamin A reserves of normal chickens were exhausted within four weeks of the chickens being placed on a vitamin A deficient ration (sd Group).

Table 11.

LIVER VITAMIN A RESERVES IN FOUR-WEEKS-OLD CHICKENS.

Group.	ds.		dss.		dd.		ss.		sd.	
	μg./g.	μg./ liver.	μg./g.	μg./ liver.	μg./g.	μg./ liver.*	μg./g.	μg./ liver.	μg./g.	μg., liver.*
Experiment 1 ..	32	166	..	..	<0·5	—	33	168	<0·5	—
	25	227	..	..	<0·5	—	11	59	<0·5	—
	13	72	..	..	<0·5	—	11	62	<0·5	—
Experiment 2 ..	22	137	..	..	<0·5	—	18	148	<0·5	—
	21	143	..	..	<0·5	—	15	103	<0·5	—
	16	115	..	..	<0·5	—	13	111	<0·5	—
Experiment 3 ..	..	..	178	984	..	..	21	125	<0·5	—
	..	..	161	1,189	..	..	21	107	<0·5	—
	..	..	134	938	..	..	18	132	<0·5	—
Experiment 4 ..	..	..	188	1,345	..	..	29	230	<0·5	—
	..	..	115	865	..	..	21	129	<0·5	—
	..	..	96	625	..	..	13	64	<0·5	—

* As levels below 0.5 μg. of vitamin A cannot be determined accurately, total liver vitamin A reserves are not given.

There were more deaths in ds Group than in sd Group, although at four weeks of age chickens in ds Group had normal liver vitamin A reserves, while those in sd Group were deficient. These greater losses in ds Group were presumably due to low initial reserves of vitamin A and the time required to build up reserves in the group. From the mortality data it appears that the chicken is more affected by vitamin A deficiency towards the end of the first week of life. There were fewer losses in dss Group than in ds Group. Presumably the high concentration of vitamin A in the ration enabled a more rapid build-up of vitamin A reserves within the first week of life. Their liver reserves of vitamin A after four weeks were certainly greater. Harvey, Parrish, Sanford and Hughes (1955), in their work on the utilization of carotene and vitamin A during the first week after hatching, established that chickens develop soon after hatching the biological systems necessary to use vitamin A.

(d) Autopsy Findings.

Five chickens in the groups on the vitamin A deficient rations had kidney lesions typical of vitamin A deficiency. There were no other lesions suggestive of vitamin A deficiency. This was not unexpected, as previous work (Gartner and Ryley 1956) showed that lesions of vitamin A deficiency are normally not apparent in chickens under six weeks of age.

(e) Weight Gains.

Chickens were weighed at hatching and at weekly intervals. Examination of weight data did not show conclusive results. This was due to the high

mortality in some groups as well as to variations in the initial weights of day-old chickens in each rearing trial. The trend was for the best weight gains to be in dss Group, followed by ss, ds, sd and dd Groups.

V. DISCUSSION.

When vitamin A is restricted in the diet, the time taken for fowls to develop clinical symptoms of vitamin A deficiency must be related to their body reserves. At an unexpectedly early stage of their experiment, Temperton and Dudley (1946) produced vitamin A deficiency in pullets after only one month on a diet supplying the equivalent of 454 I.U. of vitamin A per lb. of feed. Taylor *et al.* (1947) induced vitamin A deficiency in laying hens after four months at a feeding level of 540 I.U. of provitamin A per lb. of feed. In our 1955 pilot trial, we were unable to deplete laying hens after over seven months on a vitamin A deficient ration. In the 1956 trial, pullets on the same ration were depleted in 4½ months and again in less than two months when groups were reversed. Chemical analyses confirmed that the differences in depletion time were related to the initial liver vitamin A reserves.

The amount of vitamin A deposited in the egg must also be a function of the body reserves in the laying hen—more so than the amount of vitamin A ingested daily. After more than seven months on the deficient ration, the hens in 1955 still laid eggs containing vitamin A in amounts equivalent to that of eggs laid by hens on the vitamin A sufficient ration. If a comparison can be drawn between the hens and the pullets in the successive experiments, the vitamin A content of the eggs from the hens on the deficient ration was higher than that present in eggs from the pullets on the sufficient ration. The liver vitamin A reserves of these hens were likewise appreciably greater than the reserves of the pullets.

The second trial clearly established the effects of vitamin A deficiency in pullets. Contrary to the findings of Rubin and Bird (1942) and McClymont and Hart (1948) and in agreement with those of Temperton and Dudley (1946) and Heywang (1952), it was found that hatchability is affected by vitamin A deficiency. When chemical evidence of vitamin A deficiency was first established in the periods before and after reversal, the hatchability of the depleted pullets was 40–50 per cent., against about 80 per cent. in the controls. When field evidence of vitamin A deficiency was established, the hatchability was 10–20 per cent. As a further stage of depletion the hatchability reached 0 per cent. The egg production of the depleted pullets was also affected by vitamin A deficiency, though the time required was greater. It was 23 per cent. lower than the controls when the deficiency was first established, 57 per cent. lower when lesions of the deficiency were first apparent, and finally ceased.

The advanced stage of vitamin A deficiency in fowls is comparatively easy to detect by field examination. Administration of vitamin A in appropriate amounts will alleviate the condition and restore egg production and hatchability to normal. Incipient vitamin A deficiency is not easily recognized. Here a fall in egg production may be attributed to a seasonal decline rather than to a nutritional deficiency. As shown by the data on egg production, this decline prior to the appearance of any pathological lesions of vitamin A deficiency is appreciable and thus of considerable economic importance. The marked lowering of hatchability with incipient vitamin A deficiency may be proportionally of even greater economic importance in the case of hatcheries.

Field observations by one of us (H. W. Burton) has indicated that the egg production of pullets is less affected by any disturbance in their early stage of laying than at a later period. In D Group, there was an average of 2–4 weeks intervening between a drop in hatchability and a drop in egg production. At a later stage of laying, the intervening period averaged only 1–2 weeks in SD Group. These differences, as shown by comparison of Tables 3 and 5, may be related to those field observations.

Further observations by one of us (H. W. Burton) in connection with other poultry husbandry trials have indicated that any group of fowls has an egg-laying potential for a laying season and that when any factor which depresses production is removed, the production is thereafter enhanced in an endeavour to maintain this potential. Egg production was depressed in D Group through a deficiency of vitamin A. After reversal, DS Group consistently laid more eggs per pullet per week than SS Group. This is in keeping with the above observations.

It was postulated from the 1955 trial that the deposition of vitamin A in the egg and therefore the hatchability are functions of the liver reserves rather than of the amount of vitamin A in the diet. This appears likely for fowls with adequate reserves of vitamin A. In the 1956 trial, marginal levels of liver vitamin A were encountered in pullets on a deficient ration when the egg production and hatchability had dropped. At the end of the trial, two pullets in DS Group also had marginal liver levels, although they were on a sufficient ration and their egg production and hatchability were normal. Evidently some fowls take longer than others to build up their reserves after a depletion. Thus a knowledge of the vitamin A content of the ration is necessary for the interpretation of marginal liver levels (2 + to 10 μg./g.) in relation to production and hatchability.

From the limited amount of analytical work done on chickens at hatching, it appears that reserves of vitamin A in day-old chickens must be less than 8 μg. before the effect of the deficiency becomes apparent as the reduced hatchability.

Ewing (1947) stated that the depletion of liver vitamin A takes place at least two weeks before any pathological symptoms can be observed. From our findings, this time interval can be as great as eight weeks. This emphasizes

the usefulness of chemical analyses when no clinical symptoms are apparent in the fowls but production and husbandry data indicate a possible vitamin A deficiency. This time interval of eight weeks may be shorter and approach Ewing's estimate if the fowls are under some additional stress at the time. Increased temperatures might provide the additional stress, although the temperatures after reversal were higher than before, yet the time interval between the depletion of liver reserves and the incidence of pathological lesions was still seven weeks.

Of the 24 natural deaths in the deficient groups, four were due to partial or complete rupture of the liver. The liver has a close relationship to the venous vascular system and is formed of cells of glandular type of epithelium. It is accepted that vitamin A plays some role in the development of epithelial cells. Should vitamin A deficiency affect these cells, or cells lining the liver sinusoids, then the haemorrhages could have been due to a generalised fragmentation of these cells and spontaneous rupture. Alternatively, the fragility of the liver, as observed in eight of the 25 pullets that died in the deficient groups and in one pullet in DS Group that died 2½ weeks after reversal, would cause it to rupture when subjected to a trauma of any description. Keratinization of the epithelia is usually associated with vitamin A deficiency. However, epithelial cells with more complex physiological roles and with power to divide, such as liver cells, are not replaced by keratinizing epithelium (Wolbach 1954). Thus in the case of liver cells, keratinization would not be a mechanical hindrance to haemorrhage.

A further three deaths were due to egg peritonitis. In the ova, the follicular cells are responsible for the development of the vitelline membrane, which in turn maintains the yolk in its normal shape. Keratin is one of the components of the vitelline membrane (Romanoff and Romanoff 1949). As mentioned, keratinization of the epithelium can take place in the event of vitamin A deficiency. Should similar changes occur in the vitelline membrane and the amount of keratin be increased, it might result in the loss of elasticity and increased liability for the yolk to rupture. This could have been the cause of some of the mortality from egg peritonitis.

It appears that nodules on the oesophagus, although present prior to death, were either difficult or impossible to detect once post-mortem decomposition had set in. To examine this, a pullet from SD Group, showing nodules on the oesophagus by field examination, was killed by injecting about 10 ml. of air intravenously. It was then left for two days in the pen. Numerous nodules were seen in the pharyngeal area prior to killing it. Examination of the pharynx at autopsy revealed only three recognizable yellow nodules. The remainder of the oesophageal mucosa showed very prominent submucosal glands. These contained no yellow material, but appeared as "craters" approximately 1 mm. in diameter. Removal of the yellow material from typical nodules left similar "craters". It seems not improbable that many of the "craters"

contained nodules at death which were detached together with the general oesophageal debris by bacterial action some time after death. Thus the absence of nodules on the oesophagus in fowls showing marked post-mortem decomposition does not eliminate the possibility of vitamin A deficiency.

The data on the relationship between *Ascaridia galli* infestation and vitamin A deficiency indicate a possible relationship in the first part of the trial. However, no significant differences were found in the parasite burden after the reversal stage of the trial.

VI. ACKNOWLEDGEMENTS.

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EFFECT OF pH ON KEEPING QUALITY OF SALTED BUTTER.

By G. G. CRITTALL, Dip. Ind. Chem., A.R.A.C.I., Dairy Research Branch.

SUMMARY.

Twenty-three groups of butters were made over a period of 11 months, each group consisting of three churnings of the same cream neutralized to different pH levels. An examination of the butters after cold storage for six months showed that butters in the more alkaline range (pH 7.8–8.2) had better keeping quality.

I. INTRODUCTION.

It was noticed in butter competitions conducted over several years under the auspices of the Australian Institute of Dairy Factory Managers and Secretaries that there appeared to be a tendency for butters of high pH to have better keeping quality. This is shown in Table 1, where results of five annual competitions have been compounded. Samples which showed such abnormalities as gross underworking, high copper content and high iron content, and abnormal bacteriological counts were excluded from the data because of the possible interference of these factors with keeping quality.

It was apparent, however, that there were many variables contributing to keeping quality and in the absence of their assessment it was difficult to obtain true significance.

The apparent tendency for butters with a high pH to have better keeping quality is contrary to overseas findings. McDowall, Smith and McDowell (1937), after exhaustively reviewing the literature and conducting experiments on butters with serum pH as high as 7·7, concluded that:—

(1) Butters with high pH readings show a high incidence of "neutralizer" flavours.

(2) No improvement in keeping quality is noted when the pH is higher than 7·0.

Experiments by Barnicoat (1947) gave results which showed that, after storage at 14, 35, 45, and 65 deg. F. for various periods up to 12 months, butters of pH 7·0–7·6 with 1·5 per cent. salt were no better than butters of pH 6·6–6·8 with the same salt concentration. Over-neutralized, salted butters tended to develop rancidity after prolonged storage.

In Queensland there has been a gradual tendency in recent years towards the manufacture of butters with higher alkalinity; of the 945 butter samples examined in the routine Butter Improvement Service during the year 1954–55, 247 (26 per cent.) had pH values greater than 7·9 (quinhydrone electrode) and 366 (39 per cent.) were in the range 7·6–7·9. This means that 65 per cent. of butters had pH values greater than 7·5. In spite of these relatively high alkalinities, the incidence of "neutralizer" flavour was negligible.

II. PRELIMINARY COMMERCIAL EXPERIMENTS.

Attempts were made to perform factory trials by subdividing and neutralizing cream to give various pH ranges. It was hoped that this work would provide information regarding the effect of pH on the keeping quality of the resultant butters.

The work was carried out on the following lines:—

(*a*) At three factories with tandem or triple vacreators and a good standard of hygiene, cream was subdivided and neutralized to give acidities corresponding to butter serum pH ranges 6·8–7·2, 7·3–7·7, and 7·8–8·2.

(*b*) Separate churnings were made from each portion of cream. In the manufacture of these butters a uniform rate of salting (1·3–1·5 per cent.) was aimed at, and care was taken to ensure that all butters were well worked.

Seven groups of butters were made in this way, but it was found that practical difficulties made it almost impossible to obtain reliable results. The main difficulties met were:—

(1) Some butters had a high copper and iron content which could have been responsible for any observed difference in keeping quality. Every attempt was made to reduce the metallic contamination of the butters to the same level, but under commercial factory conditions this was not possible.

(2) The attainment of the desired pH was found to be very difficult when using normal types of cream neutralizer. Much greater amounts of neutralizer than would be expected theoretically were found necessary to achieve a pH in the higher alkaline ranges, due probably to the buffering capacity of the cream or to the partial saponification of the fats.

It thus became apparent that a change in experimental technique was necessary in order to overcome the interference due to these factors.

III. EXPERIMENTAL CHURNINGS.

In the later work, smaller quantities of cream were used in one plant only, under circumstances which permitted the control of all factors which were found to interfere with the earlier experiments. Neutralization to give the desired pH in the final butter serum was obtained by preliminary acidity correction to 0·12 per cent. with normal commercial neutralizers, followed by pasteurization and subsequent adjustments with sodium hydroxide to give final acidities. In this way the buffering capacity of the cream was overcome.

Twenty-three groups of churnings were made and boxes of the butter examined after six months' cold storage at 10 deg. F. Three pats were also taken from each churning; two were examined fresh and one after storage for four weeks at approximately 50 deg. F. In order to provide information on the losses of butterfat in churning, fat tests were performed on cream samples taken from the churns and on the corresponding buttermilks.

(1) Methods.

(a) General.

The experiments were conducted at regular intervals from April 1954 to March 1955 at the Queensland Agricultural High School and College, Lawes. The cream used was a blend of choice and first quality, and the initial acidities varied from 0·16 to 0·47 per cent. Approximately 250 gal. of cream was neutralized to 0·12 per cent. acidity with sodium sesquicarbonate or sodium bicarbonate, pasteurized in a "Junior" vacreator at approximately 200 deg. F. (vacuums 14 and 27 in., water temperature rise 40 deg. F.), cooled in a heat exchanger, and held overnight in a stainless steel holding vat.

Prior to churning, all the cream was neutralized with 10N sodium hydroxide to 0·10 per cent., and one-third of this treated cream was churned. The remaining two-thirds of the cream was further neutralized with sodium hydroxide to approximately 0·04 per cent. and half this quantity was churned. Sodium hydroxide neutralizing solution was then added to the balance until it gave a faint pink colour with phenolphthalein indicator. This cream was then run into the same churn as was used for the previous two portions and churned in a similar manner. In this way three comparative churnings were made, each yielding about six boxes of butter. No break-water was used during churning, and efforts were made to keep the moisture and salt content uniform in each group.

Three pats were hand-cut from the butter during packing, two being used for grading and analyses while fresh, and one being stored at 50–55 deg. F. for one month. A box of 56 lb. from each churn was held in cold storage at 5–10 deg. F. for six months before examination.

(b) Analytical.

pH estimations were made on the centrifuged serum, using both a glass electrode and a quinhydrone electrode.

Peroxide values were determined, using the ferric thiocyanate method of Loftus Hills and Thiel (1946), with a chloroform-methanol solvent and a photoelectric colorimeter.

Moisture and salt were determined by the Kohman (1919) method.

Bacteriological analyses were carried out on duplicate samples by methods specified by Muller and Nichols (1950).

Microscopic examination, to give a measure of butter working by estimation of the number of moisture droplets larger than 30μ in diameter, was carried out, using the method of Muller (1952).

Copper and iron were estimated by the wet-ashing method of McDowell (1947).

(2) Results.

(a) Butter Flavour.

The losses in flavour points of butter in the three pH ranges are set out in Tables 1 and 2. These results show a marked trend in flavour score in that the neutral butters lost three times as many points as the very alkaline butters, and the slightly alkaline butters lost twice as many as the very alkaline butters. These differences were apparent after four weeks in storage and were maintained after six months' storage. It is significant that the neutral butters lost an average of more than three full points for flavour over the six months' storage period.

Table 1.

Comparison Between pH and Loss in Flavour Score After Cold Storage for Three Months. (Competition Butters.)

	pH Range (Glass Electrode).		
	6·8–7·2.	7·3–7·7.	7·8–8·2.
No. of Samples	48	61	44
Average Loss in Flavour Points	0·67	0·44	0·31

Table 2.

Influence of pH on Butter Flavour after Cold Storage.

	pH Range (Glass Electrode).		
	6·8–7·2.	7·3–7·7.	7·8–8·2.
Average Loss in Flavour Score After 4 Weeks (pat)	2·3	1·5	0·8
Average Loss in Flavour Score after 6 months (box)	3·1	2·0	1·0

(b) Fat Losses.

Table 3 gives the results of butterfat estimations on the cream samples taken from 15 churnings and their corresponding buttermilks. Most of the fat tests on cream were determined by volumetric analysis, which, though not as accurate as the gravimetric method, does provide a useful guide. Although

Table 3.

Influence of pH on Butterfat Losses.

	pH Range (Glass Electrode).		
	6·8–7·2.	7·3–7·7.	7·8–8·2.
Average Fat Percentage in Creams	32·9	32·1	30·9
Average Percentage Fat Losses in Buttermilks	1·45	1·40	1·67

there was some evidence of a slightly greater dilution in the lower testing, more alkaline creams, appreciable differences in fat losses were recorded; these appeared to be related to pH. The fat losses were calculated from the Udy formula (McDowall 1953), and varied from 1·45 per cent. for nearly neutral creams to 1·67 per cent. for very alkaline creams.

(c) Peroxide Values.

Metallic contamination was in the ranges 0·10–0·20 p.p.m. copper and 0·7–1·5 p.p.m. iron. Peroxide values of butter of various pH values after six months' storage are shown in Table 4. No explanation of the wide range in peroxide values is offered. No correlation is apparent between pH and degree of oxidation. These findings confirm those of Muller and Ferricks (1953).

Table 4.

Comparison of pH and Peroxide Values.

	pH Range (Glass Electrode).						
	6·5–6·7.	6·8–7·0.	7·1–7·3.	7·4–7·6.	7·7–7·9.	8·0–8·2.	8·3–8·4.
No. of Samples	9	11	10	15	4	11	6
Range of Peroxide Values	·08–·52	·13–·63	·09–·50	·08–·45	·21–·35	·11–·32	·10–·32
Average Peroxide Value ..	0·22	0·28	0·25	0·25	0·28	0·21	0·20

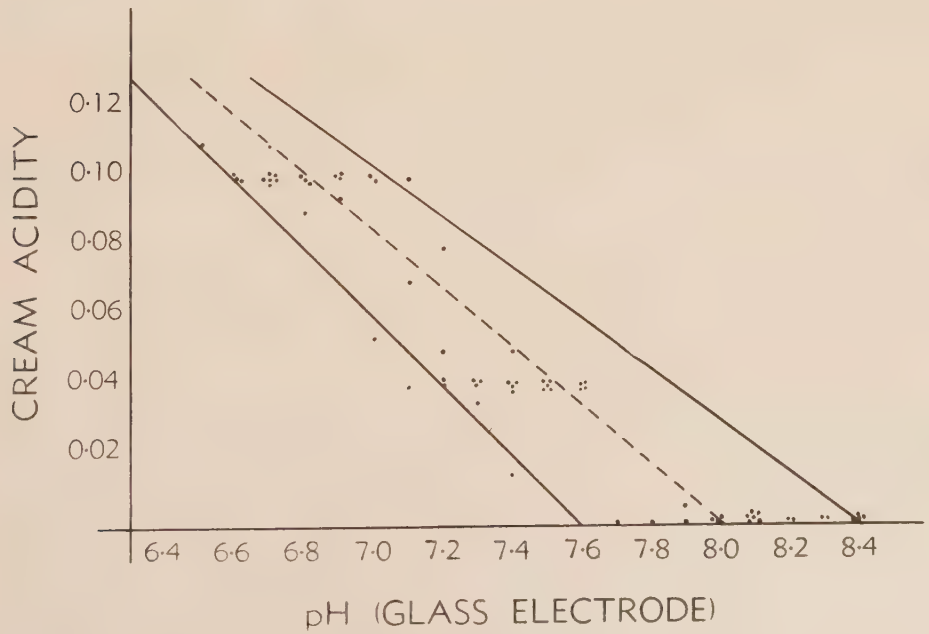

Fig. 1.

Relationship Between Final Cream Acidity and pH of the Butter Serum.

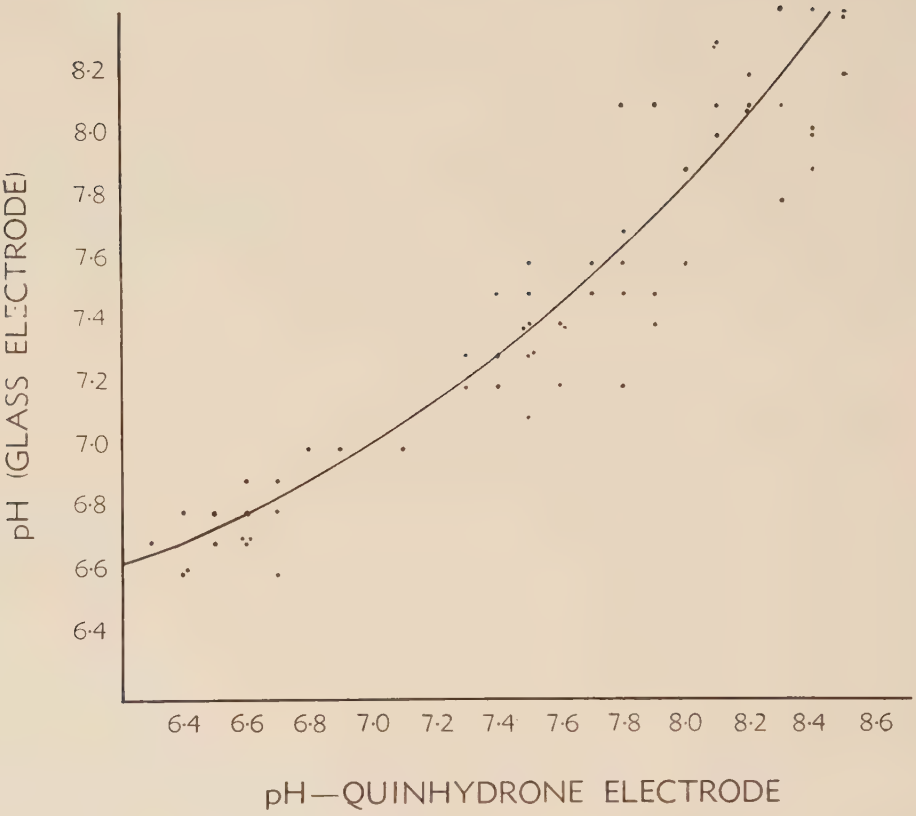

Fig. 2.

Relationship Between pH Determined by the Glass Electrode and pH Determined by the Quinhydrone Electrode.

(d) pH of Butter Serum.

The difficulties of neutralization to a desired pH level can be seen in Fig. 1. Cream acidities of 0·10 per cent. resulted in butters with serum pH readings varying from 6·6 to 7·1; in the highly alkaline ranges the variation is even greater.

pH determinations were made on all butters by using both glass and quinhydrone electrodes. The relation between the two is shown in Fig. 2. The quinhydrone electrode gives results fairly comparable with the glass electrode in the pH range 7·0–7·8, but results are somewhat lower than the glass electrode in the lower ranges and slightly higher in the more alkaline ranges.

(e) Bacteriological.

No correlation between pH and bacterial development after storage was evident. With butters in the low pH range a significant increase in plate count on milk agar was found in three cases, and in three other cases there was a significant decrease. In the case of butters in the alkaline ranges, five showed an increase in bacterial population after storage, and five a decrease.

(3) Discussion.

The results show a better keeping quality for salted butters in the alkaline pH range. It is significant that butters in this range lost an average of only one point for flavour after prolonged storage, while butters in the slightly alkaline and neutral ranges lost an average of 2 and 3 points, respectively. As good keeping quality is desirable for the United Kingdom market, it would seem preferable to produce a butter of high alkalinity, to stand up to the long storage periods necessary during shipment, provided, of course, that the butter is of sound quality initially. However, it also appears that some increases in fat losses occur during the manufacture of highly alkaline butter, and the extent of these losses may well militate against such manufacture.

It was thought before these experiments were commenced that severe neutralization might destroy some of the typical butter flavour, but no apparent significant difference in initial quality was found by the graders. The average flavour scores for the low, medium and high pH ranges were 92·1, 92·2 and 92·3 respectively. One case of "soda" flavour was noticed, but this was very slight and no loss in points score was sustained.

It is recognized that neutralization with sodium hydroxide is not a usual commercial practice. This alkali was used during the experiments for the sole purpose of ensuring more accurate neutralization for the higher pH ranges. The results did show an appreciable benefit in keeping quality for butters neutralized to "zero" with sodium hydroxide, and experiments are now being performed to find out if the usual neutralizing agents have a similar effect.

IV. ACKNOWLEDGEMENTS.

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CHEESE YIELD INVESTIGATIONS: THE SAMPLING OF CHEESE MILK.

By V. R. SMYTHE, M.Sc.Agr. (Senior Bacteriologist), and N. B. STANTON, B.Sc., A.R.A.C.I (Analyst), Dairy Research Laboratory.

SUMMARY.

Some methods of sampling cheese milk, including those commonly used in Queensland, were examined. The results obtained by these methods have been compared with those obtained by using an experimental sampler designed and made for the purpose. The fat content of a series of samples has been taken as the criterion of sampling efficiency.

Multiple samples at all levels on each side of the vat were used to provide a "fat standard" for each vat. The experimental sampler gave results which agreed most closely with the standard, but a sample composed of 12 equal quantities of milk from 12 positions on the surface of the vat, six from each side, also gave a good result. In normal factory practice starter is added to the vat and a correction for this must be made to the result given by the experimental sample.

A "drip" sample obtained in a bucket hung under a loosened pipe joint gave a very unreliable sample.

The construction of the experimental sampler is outlined.

I. INTRODUCTION.

In the course of investigations into the cheese-yielding capacity of milk the need has arisen for an accurate sample of the milk entering any particular cheese vat. Without such a sample any work done along the lines of milk composition and cheese yields is rendered worthless. Veale (1929) and McDowall (1936) earlier gave attention to this matter.

In this paper are presented the results of an enquiry into some methods of sampling, together with the results obtained with a new sampling apparatus.

Methods previously employed in this State for the sampling of bulk cheese milk have included:—

(1) Taking a dipperful of milk from one or more points on the surface of the vat.

(2) Drip sampling from a loosened nut in the milk pipe-line.

(3) Drip sampling from a hole in the milk pipe or flue leading to the cheese vat.

The two drip samples appear to suffer from the disadvantage that the rate of flow of milk as a sample is inconsistent with the rate of flow of the bulk of the milk. Milk lying in the pipe or flue when flow ceases runs through the loose nut or hole and becomes part of the sample. In the case of a horizontal milk line a large part of the sample may consist of milk lying in the pipe during

Fig. 1.

End View of Experimental In-line Sampler.

periods of pasteurizer shut-down. Observations have shown that the loosened nut is open to a further objection in that the rate of sampling fluctuates greatly even during a continuous run.

It was obvious that a method of sampling was required which would give a constant rate of sampling dependent on pumping rate and which did not have to be continually watched.

II. METHODS.

In normal cheese factory practice, starter is added to the milk in the vat prior to the vat becoming full. Thus a sample of the milk in the vat when full will contain starter, which is usually skim-milk and which will cause a slight but significant lowering of the fat content (approx. 0·1 per cent.). For our purpose where samples have to be transported to the laboratory for detailed analysis, the presence of starter is objectionable for a further reason in that the acidity, which develops rapidly, becomes a hazard. Consequently, it was necessary to devise some means of accurately sampling the milk prior to its entering the vat.

In order to fulfil this purpose the sampling device shown in Figs. 1 and 2 was constructed. Primarily, it consists of a short coupling of $1\frac{1}{2}$ in. stainless steel which can be attached at any union of the milk pipe-line from the pasteurizer to the vat. Through the upper wall of the coupling a metal tube of $\frac{1}{8}$ in. bore projects downward into the lumen of the pipe. The end of this tube is truncated so that the tube opening faces into the milk flow and the height of the tube can be adjusted to the approximate centre of the pipe-line. Attached to the other end of the tube is a small cock which enables the sampling rate to be adjusted. A simple hook hangs from the sampler to hold the sample container—usually a bucket. The whole apparatus can be dismantled for cleaning and can be sterilized before use.

In practice, no flow of milk can be obtained from this sampler unless the milk pump is operating and creating sufficient milk pressure to force milk up the curved tube. When the pump is switched off, the milk level in the line almost immediately falls below the truncated end of the sampling tube and no sampling occurs. Thus milk which is residual in a line after the pump has been switched off does not continue to be sampled.

(1) Testing of Sampler.

In testing this sampling device the fat content of the sample was used as the criterion of sampling accuracy, to which use it would appear to be pre-eminently suited. The need arose, however, for obtaining a standard fat assessment against which that obtained from the sampler could be judged. Such a standard was obtained in the following manner.

Fig. 2.

Side View of Experimental In-line Sampler.

The vat stirrers were set in motion during the whole time milk was entering the vat and were kept in motion until sampling ceased. One minute after the vat was full, six samples were taken, viz.,

(1) A dipperful was taken from each of six positions along the surface of the vat along one side.

(2) Same as (1) along the other side.

(3) A stainless-steel sampling tube capable of reaching the bottom of the vat was used. The height of milk at the side of the vat was measured, and at half this distance from the bottom of the tube a metal band $\frac{3}{8}$ in. wide was fixed in place by means of a milled-head screw. The thumb was placed over the end of the tube, the tube immersed to the point of the metal band, and the thumb released for a few seconds to allow the level of the milk in the tube to become steady. The thumb was then placed over the end of the tube again, the tube withdrawn from the vat, and the milk so removed allowed to run into a bottle. This procedure was followed for six positions along one side of the vat.

(4) Sampling as in (3) was repeated for the other side of the vat.

(5) The sampling tube was immersed to the full depth of the milk, using the same procedure for removing the sample as was adopted in taking sample 3. Again, milk was taken from six positions along one side of the vat.

(6) The above (sample 5) was repeated for the other side of the vat.

In order to standardise techniques, in every run of this experiment one of us took samples 1 and 2 while the other took samples 3, 4, 5 and 6.

The fat content of each of the six samples above was measured in quadruplicate. The mean of all 24 estimations thus obtained, calculated to the second place of decimals, has been used as the standard fat content of the vat.

(2) Examination of Other Sampling Methods.

For purposes of comparing samples obtained by means of the experimental sampler with those taken by other methods, two additional samples were taken for each vat examined, viz.,

(1) One was taken from the same pipe-line as the experimental sampler, and in a position adjacent to but nearer to the vat. A nut was loosened in the line to provide a drip sample.

(2) With the stirrers still in motion, a dipperful of milk was taken at each of 12 positions from the surface of the vat. For convenience, this sample is termed the field sample.

For all three samples (experimental sampler, drip and field), fat estimations were performed in eight replications and the means calculated to two places of decimals.

During each factory visit two vats of milk were examined. In some vats no starter was added until all sampling was completed; in others, starter was added in the normal way. When starter was added corrections were made from a knowledge of the weight of milk in the vat and weight of skim-milk, as starter, added to allow for the difference this would make to the fat determination of the standard and of the field sample. When taking cheese yields into account, the correction of course has to be made in the opposite way, and the analysis of a sample of milk before the addition of starter has to be corrected for this starter when considering the yield of cheese of a particular vat.

All fat determinations were made by the Babcock method, giving readings to 0·1 per cent. In this testing the same 17·6 ml. pipette was used throughout. The operations of pipetting the samples, adding the acid, etc., were standardised so that the same worker carried out the same operations on every sub-sample. This was rigidly adhered to so that variations due to differences in techniques of different workers would be eliminated.

III. RESULTS AND DISCUSSION.

In all, 10 vats were examined, six at Factory A and four at Factory B. A variation of 0·3 per cent. fat between eight replicate Babcock tests occurred only once. A variation of 0·2 per cent. fat between similar replications was not uncommon. Such a variation between replicate fat tests is of some importance in view of the precautions which were taken to standardise techniques in Babcock testing. Thus it would appear necessary in all cheese yield work to perform the fat tests at least in quadruplicate in order to minimise error from this source. McDowall (1936) drew attention to the variation in the apparent yield of cheese which results from an error of $\pm$ 0·1 per cent. (0·2 per cent. variation) in the fat test. The results as means are summarised in Table 1.

The divergence of the value for the experimental sampler from the standard varied from 0·01 per cent. to 0·06 per cent., the mean being 0·03 per cent. (S.D. $= \pm$ 0·028). This must be considered very satisfactory.

The divergence of the value for the field sample from the standard varied from 0·01 to 0·09 per cent., the average being 0·05 per cent. (S.D. $= \pm$ 0·039). This must be considered fairly satisfactory and entirely adequate for the needs of routine vat testing by the factories themselves.

Table 1.

COMPARISON OF SAMPLING METHODS.

Factory.	Date.	Vat No.	Starter Added.	Fat Percentage.			
				Vat Standard.	Experimental Sampler.	Drip Sample.	Field Sample.
A	29–9–54	1	+	3·92	3·95	3·90	3·90
		2	—	4·14	4·10	4·25	4·23
	27–10–54	1	—	4·20	4·16	4·13	4·19
		2	+	4·11	4·05	4·11	4·08
	17–11–54	1	—	4·06	4·04	4·05	4·10
		2	+	4·09	4·11	4·38	4·13
B	9–12–54	1	+	4·27	4·26	4·30	4·24
		2	+	4·11	4·08	3·91	4·09
	14–12–54	1	—	3·94	3·91	3·96	3·95
		2	—	4·04	4·03	4·06	4·03

+ = Starter added and correction made.
— = Sampled before starter added.

The divergence of the value for the drip sample from the standard varied from 0·00 to 0·29 per cent., including values of 0·11 per cent. and 0·20 per cent. The average was 0·08 per cent. (S.D. = ± 0·125). This result would indicate that this method of sampling is unreliable, and its use is to be discouraged.

The results obtained were also examined in order to discern any differences in fat percentages in milk from different levels in the vats and from different sides of the vat. The mean values for 10 vats were as follows:—

Table 2.

Sample.	Mean Fat Percentage (10 Vats).
Surface, south side	4·01
Surface, north side	4·02
Middle, south side	4·035
Middle, north side	4·00
Bottom, south side	4·08
Bottom, north side	4·045

It is apparent from these results that there was no pattern of difference in fat percentage between the top, middle and bottom layers of milk, or in the milk from either side of the vat. The only conclusion possible from this finding is that the agitators, being in motion for the whole time the vats were filling with milk, provide very thorough mixing.

IV. ACKNOWLEDGEMENTS.

Thanks are due to the Pittsworth and Southbrook Co-operative Dairy Associations for assisting in the performance of these experiments and to Mr. J. Zerner, manager of the Pittsworth Association, for useful advice and criticism. Thanks are also due to Mr. P. B. McGovern (Departmental Biometrician) for help in the statistical interpretation of results.

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INHIBITORY FACTORS IN CHEESE MILK.

By AILSA GILLIES, B.Sc. (App.), Dairy Research Laboratory, Division of Dairying.

SUMMARY.

Some milks were found to be inhibitory to one group of starters when tested by a 6½ hour activity test, but the phenomenon was not evident when the same milk was tested by a 6½ hour vitality test in which the milk is clotted with rennet.

Comparison of these tests with actual vat workings at a commercial cheese factory showed that the results of vitality testing correlate well with acidity production in the vat. In contrast, the results obtained from the activity test are markedly affected by inhibitory factors in the milk and show no correlation whatever with vat workings.

It is concluded that the activity test in pasteurized milk is a very unreliable measure of the ability of starter strains to work satisfactorily in the cheese vat and that the vitality test should be used for this purpose.

I. INTRODUCTION.

In the past few years, both in Australia and overseas, attention has been given to the so-called inhibitory winter milk supplies. Two sets of workers —Czulak and Meanwell (1951) in England and Jago (1954) in Australia— have found that, when grown in pasteurized milk for 6 hours at 30 deg. C., single strain starters fall into two categories on the basis of their acid production. One group comprises those starters which are inhibited in pasteurized milks; the other contains those that are unaffected. Both workers also found that sterilizing the milk eliminates the inhibitory effect.

In Queensland, the New Zealand vitality test (Whitehead and Cox 1932) has been used as a means of gauging acid-producing activity and no general incidence of inhibitory milk has been noticed with this test. It was only when using the activity test (Anderson and Meanwell 1942) as a quicker and less tedious method of examining acid-producing abilities that any evidence of an inhibitory phenomenon became apparent.

The two tests under consideration measure different things. The activity test is a measure of acidity production in heated or raw milk, whereas the vitality test measures acid production after the addition of rennet to the milk.

The present work was designed to establish any differences that may result when comparing acid production in the two tests. If these differences are marked, one or other of the tests cannot be used to predict the behaviour of starters in the vat.

II. LABORATORY COMPARISON OF TESTS.

(1) Methods.

In order to obtain a representative range of factory milks the bulk supply to four different factories close to the laboratory was sampled. In some cases mixed evening and morning milk was used and in some cases milk was held chilled at the factory overnight before it was pasteurized. All milks were sampled as soon as they were H.T.S.T. pasteurized, and testing was commenced immediately they were received at the laboratory. Each milk was tested in duplicate by both activity and vitality tests.

Six starters were tested. They divided into two groups: (a) those which were inhibited when cultured in pasteurized milk; and (b) those which were resistant to the inhibitory principle in the same milk. The six starters chosen were: (*a*) HP, R6, R1 (New Zealand strains), and (*b*) C1, C2, C13 (C.S.I.R.O. Laboratory, Victoria). All starters were active in sterilized milk and were quite distinct with regard to phage relationships. It is to be noted that strain C2 is a strain of *Streptococcus lactis;* all others are strains of *S. cremoris.*

The activity test as employed by Czulak and Meanwell (1951) and Jago (1954) was adapted so that 50 ml. of milk was dispensed in 2 oz. McCartney bottles, 0·5 ml. of starter added to each bottle, and the bottles incubated at 30 deg. C. for 6½ hours. Acidity was determined at 5½ hours and 6½ hours.

The vitality test was based on that of Whitehead and Cox (1932) and was as follows:—

One pint of milk at 86 deg. F. was placed in each jar, 6 ml. of starter added, and the jars placed in a water bath at 86 deg. F.

After 1 *hour,* diluted rennet was added in appropriate concentrations to give a clot in 15 min. and mixed thoroughly.

After 1½ *hours,* the curd was cut as evenly as possible in two directions and the temperature raised to cooking temperature (100 deg. F.) over a period of 30 min.

After 3½ *hours,* the whey was drained off. The temperature of the bath and contents was then allowed to fall to 86 deg F.

After 5½ *hours,* the whey was drained and the acidity determined.

After 6½ hours, the whey was again drained and the acidity determined.

The vitality test has been used in Queensland because the conditions of the test approximate closely the cheese-making process. The starters were cultured at 86 deg. F. for the greater part of the test and were raised to 100 deg. F. only for a time comparable with cooking in the cheese vat. Thereafter, the temperature was allowed to fall back steadily to approximately 86 deg. F. A somewhat similar falling back occurs in practical cheese-making.

In both tests acidity was determined by titrating 9 ml. whey (vitality test) or 9 ml. milk (activity test) with O·1N NaOH, using phenolphthalein as indicator.

(2) Results.

Acidity determinations for both activity and vitality tests were obtained from titrations done after 5½ hours and 6½ hours. The rise in the final hour has been noted for both sets of tests in Tables 1 and 2.

Table 1.

Acidity Production in One Milk Using the Activity and Vitality Tests.

Starter.	Activity Test. Acidity Percentages.			Vitality Test. Acidity Percentages.		
	5½ hr.	6½ hr.	Rise in Final Hour.	5½ hr.	6½ hr.	Rise in Final Hour.
HP	·22	·28	·06	·34	·59	·25
R6	·22	·29	·07	·41	·67	·26
R1	·24	·33	·09	·34	·58	·24
C1	·35	·49	·14	·32	·46	·14
C2	·29	·50	·21	·36	·60	·24
C13	·34	·53	·19	·32	·52	·20

Table 2.

Average Acidity Production of Six Milks Using the Activity and Vitality Tests.

Starter.	Activity Test. Acidity Percentages.			Vitality Test. Acidity Percentages.		
	5½ hr.	6½ hr.	Rise in Final Hour.	5½ hr.	6½ hr.	Rise in Final Hour.
HP	·22	·26	·04	·34	·46	·12
R6	·23	·29	·06	·43	·56	·13
R1	·24	·30	·06	·36	·46	·10
C1	·36	·47	·11	·34	·45	·11
C2	·34	·51	·17	·38	·56	·18
C13	·35	·51	·16	·33	·46	·13

In both tests there were instances of variation between titrations of duplicate tests. In the vitality test 75 per cent. of duplicate titrations showed a difference of ·02 per cent.; in the activity test 77·7 per cent showed a difference of ·01 per cent. or less. In all instances, the mean value was taken as the true one. A typical set of results is given in Table 1.

The averages of results from tests carried out on six different days are set out in Table 2.

These results clearly demonstrate that there were marked differences in the acidity production of some strains in the two tests. These differences are such that the activity test would tend to give a false impression of the acid-producing ability of these strains under conditions of renneting. It is unlikely, therefore, that this test would be an accurate guide to vat workings.

III. FACTORY COMPARISON OF TESTS.

Following the results obtained in the laboratory when comparing the activity and vitality tests, it was decided to extend the work to factory conditions and compare starter behaviour in the cheese vat with both tests.

Accordingly, arrangements were made with one Dairy Association to use only one single strain starter in the vat on the days when trial tests would be run.

(1) Methods.

For each series of tests a drip sample from a sterilized sampler was taken so that a representative sample of the milk in the vat was obtained. The milk was collected in a sterile can and the milk immediately transported to the laboratory, where tests were started immediately.

For each day the tests were run the cheesemaker kept complete vat records. In addition, a sample of the bulk starter used in the vat was collected and brought back to the laboratory for testing with the laboratory starters. The work was so arranged that one of the same six starters used in the laboratory test was used in the vat. When the milk samples arrived at the laboratory, sub-samples were taken for vitality and activity tests.

(2) Results.

The results representing tests run on two days are set out in Tables 3 and 4.

It is obvious from these results that strain R6 works satisfactorily in the vat even though it shows little activity as judged by the activity test. On the other hand, strain C13 gave satisfactory acid production in the cheese vat and during both tests. This strain was apparently resistant to the inhibitory factor in pasteurized milk and its vat behaviour correlates well with results of the activity test. One may conclude that the activity test is not a reliable indicator of the acid-producing ability of a cheese starter in the vat.

These results clearly demonstrate also that there were marked differences in the acidity production in the two tests. These variations are such that the activity test tends to give a false impression of the acid-producing ability of many strains under conditions of renneting. It is unlikely, therefore, that this test would be an accurate guide to acid production under normal cheese-making conditions.

Table 3.

ACID PRODUCTION OF STARTERS IN PASTEURIZED MILK USING (*a*) ACTIVITY TEST, (*b*) VITALITY TEST, AND (*c*) COMMERCIAL CHEESE-MAKING CONDITIONS.

Starter.	Activity Test. Acidity Percentages.			Vitality Test. Acidity Percentages.			Vat Records (C13 Starter used 1·9%).		
	5½ hr.	6½ hr.	Rise in Final hour.	5½ hr.	6½ hr.	Rise in Final hour.	Stage.	Acidity.	Temperature.
							Starter added (at start)		
HP	·21	·24	·03	·27	·47	·20	Set (7 min. after start))	·185	88°F.
R6	·21	·23	·02	·42	·64	·22	Cut (37 min. after start)	·13	
R1	·25	·30	·05	·32	·51	·19	Cooked	·13	100°F.
C1	·37	·50	·13	·37	·50	·13	Run off (2 hr. 17 min. after start) ..	·16	
C2	·40	·52	·12	·32	·46	·14	Dry	·21	
C13	·32	·45	·13	·27	·46	·19	Milled (4 hr. 17 min. after start) ..	·62	
C13 B.S.* ..	·39	·54	·15	·33	·51	·18	Salted (4 hr. 42 min. after start) ..	·65	

* B.S. = Bulk starter.

Table 4.

ACID PRODUCTION OF STARTERS IN PASTEURIZED MILK USING (*a*) ACTIVITY TEST, (*b*) VITALITY TEST, AND (*c*) COMMERCIAL CHEESE-MAKING CONDITIONS.

Starter.	Activity Test. Acidity Percentages.			Vitality Test. Acidity Percentages.			Vat Records (R6 Starter used 1·3%).		
	5½ hr.	6½ hr.	Rise in Final hour.	5½ hr.	6½ hr.	Rise in Final hour.	Stage.	Acidity.	Temperature.
							Starter added (at start)		
HP	·23	·25	·02	·30	·52	·22	Set (6 min. after start)	·185	88° F.
R6	·22	·25	·03	·40	·62	·22	Cut (35 min. after start)	·13	
R1	·24	·25	·01	·30	·55	·25	Cooked	·13	100° F.
C1	·30	·41	·11	·35	·56	·21	Run off (2 hr. 10 min. after start) ..	·16	
C2	·36	·43	·07	·34	·52	·18	Dry	·22	
C13	·29	·40	·11	·32	·50	·18	Milled (3 hr. 55 min. after start) ..	·65	
R6 B.S.* ..	·25	·29	·04	·37	·60	·23	Salted (4 hr. 25 min. after start) ..	·70	

* B.S. = Bulk starter.

IV. EFFECT OF TEMPERATURE.

It was thought possible that the difference in results obtained from the two tests might be explainable on a temperature basis because the tests are not performed at the same temperature. Tests were conducted in order to examine this possibility.

(1) Methods.

Pasteurized milk was dispensed in 50 ml. quantities into McCartney bottles and 1 per cent. of starter added to each bottle. Starters HP, R6, R1, C1, C2, C13 were used and all tests were carried out in duplicate. Two series of bottles of milk were inoculated. Series A was incubated at 30 deg. C. for 6½ hours, and Series B was placed in a water bath at vitality test temperatures for 6½ hours.

(2) Results.

Acidity production in one milk, using (*a*) an incubation temperature of 30 deg. C. for 6½ hours (Series A), and (*b*) a temperature of 37 deg. C. for 2 hours during the course of 6½ hours' incubation (Series B), is shown in Table 5.

From these results it is evident that there is little effect due to the temperature difference. It is clear also that the starter strains again fall into two distinct groups with regard to acidity production.

Table 5.

EFFECT OF INCUBATION TEMPERATURE.

Starter.	Series A. Acidity Percentages.			Series B. Acidity Percentages.		
	5½ hr.	6½ hr.	Rise in Final Hour.	5½ hr.	6½ hr.	Rise in Final Hour.
HP	·26	·30	·04	·23	·27	·04
R6	·28	·30	·02	·28	·30	·02
R1	·28	·30	·02	·30	·34	·04
C1	·58	·67	·09	·61	·64	·03
C2	·55	·68	·13	·54	·70	·16
C13	·58	·68	·10	·50	·67	·17

V. DISCUSSION.

The experiments reported herein were undertaken to compare the acid-producing abilities of starter cultures in the activity test and the vitality test, and the relationship of the results of these tests to starter behaviour in the cheese vat.

When the activity test was used to determine the acid production of the cultures, it was found that the starters were divided into two groups—the fast acid producers, and strains which were susceptible to some inhibitory factor in the pasteurized milk. This finding is similar to that of Czulak and Meanwell (1951) and Jago (1954). These workers divided their starter cultures into susceptible and resistant groups and used the resistant types in cheese-making to overcome the inhibition which had been found to cause marked vat slowness. However, the inhibitory principle which was investigated by these workers apparently differs in some way from that detected by the activity test in the present work, even if only in degree; for it was found that, although a culture was inhibited in the activity test, acid production in the cheese vat was quite normal.

When a vitality test was conducted using pasteurized milk which inhibited some starter strains in the activity test, all starters produced normal amounts of acid and there was no evidence of any inhibitory factor. It was also found that the vitality test results in the laboratory correlated very well with commercial vat workings, and that the inhibitory factor revealed by the activity test was without effect when rennet was added, as in the vitality test and in cheese manufacture. Therefore, it is apparent that the activity test in pasteurized milk may be a most unreliable indicator of the suitability of a starter for cheese-making, and it should not be used as a basis for starter strain selection. The selection of strains on the basis of resistance to an inhibitory principle, such as was present in the pasteurized milk used in these investigations, is not justified. The vitality test is a more satisfactory means of selecting or differentiating starter strains suitable for cheese-making.

No attempt has been made in the present work to study the exact nature of the inhibitory factor in milk, but it is suggested that the inhibition may be a manifestation of the natural bactericidal property of milk, which retains greater activity during the cooler winter weather and quickly passes off under the high temperatures of the Queensland summer.

It was considered that the differences between the results of the activity and vitality tests using pasteurized milk might be due, at least in part, to differences in the temperatures at which the tests are conducted. However, it has been found that this was not the case, and, consequently, it would seem that the suppression of the inhibitory principle in the vitality tests and in the cheese vat is a result of the clotting of the milk with rennet.

VI. ACKNOWLEDGEMENT.

It is desired to thank the manager of the Pittsworth Co-operative Dairy Association, Pittsworth, for his co-operation in keeping vat records.

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A RIPE FRUIT ROT OF THE STRAWBERRY CAUSED BY A SPECIES OF *GLOEOSPORIUM*.

By O. W. STURGESS, M.Agr.Sc. (Formerly Pathologist, Science Branch.)

SUMMARY.

A species of Gloeosporium **causing a ripe fruit rot of the strawberry is described.**

During the spring and summer, seasonal temperatures beyond a mean daily temperature of 65 deg. F. are shown to be an important factor influencing economic levels of fruit wastage in diseased strawberry plantings.

I. INTRODUCTION.

Within horticultural districts surrounding Brisbane, commercial strawberry plantings have suffered a varying wastage of ripe fruit due to a species of *Gloeosporium* (Sturgess 1954). The disease was recorded in the first instance by R. B. Morwood within a strawberry clonal trial at Redlands Experiment Station, Ormiston, during the spring months of 1951. It was not until the following year that serious fruit losses were observed; these were in a commercial planting at Manly. Further losses occurred in many other strawberry-growing areas around Brisbane during the spring and early summer months of the next three years.

No similar ripe fruit rot of the strawberry appears to have been recorded. On reviewing the literature, it was noted that two species of the closely related form genera, *Gloeosporium* and *Colletotrichum,* have been described as strawberry pathogens. One, *Gloeosporium fragariae* (Lib) Mont., was briefly described by Saccardo (1884). It has caused a strawberry leaf spot disease in France, Great Britain, Belgium, Germany and North America. The other is *Colletotrichum fragariae* Brooks, which produced a leaf anthracnose and wilt condition of the strawberry in Florida (Brooks 1931, 1933). Descriptions of the two diseases and the morphology of their causal organisms do not conform to those of the ripe fruit rot disease.

II. DESCRIPTION OF THE DISEASE.

Under field conditions, the early formation of the rot, which is restricted to mature strawberry fruit, is characterized by the development of one or more tan or light-brown, water-soaked lesions. Shrivelling of affected tissues which is associated with the mycelial penetration of the fruit results in a compact depressed lesion, together with an intensification of the colour from tan to dark-brown. This is followed by the eruption of pink spore masses from acervuli immediately below the surface. With further deterioration the diseased fruit remains firm and the end-point is complete mummification. Occasionally a soft breakdown occurs, owing to the entry of secondary soft rot organisms.

Strawberry stolons, leaf stalks, peduncles and pedicels are also affected by the disease. The symptoms on these appear as black, slightly depressed, longitudinal lesions.

III. ECONOMIC SIGNIFICANCE OF THE DISEASE.

The strawberry cropping season may be broadly divided into three phases of production, depending on local seasonal conditions (Fig. 4).

During the first phase the plants set a bulk of large fruit during late winter, with a production peak in the early spring. The second phase consists of a short period of poor flowering and fruit set which is confined to the mid-spring section of the cropping period. During the third phase, which extends from late spring to midsummer, the plants flower profusely and set a large number of small strawberries which are classed as jam berries. The fruit production rises to a second peak during early summer. Following the development of stolons and runner plants from parent plants, fruit production tapers off during midsummer.

In diseased plantings the large winter strawberries, which are sent to local and interstate markets or to canning factories, are free from the ripe fruit rot. Only the smaller jam strawberries, which are set during the second and third phases of production, are subject to fruit wastage from this disease.

In spite of the fact that it is the late-season factory fruit which is mostly affected, the disease may reach a level of economic importance. A number of individual fruit counts and disease estimates illustrate the seriousness of the disease during the later stages of fruit production (Tables 1 and 8). For example, at Ormiston on Oct. 28, 1952, 33 per cent. of the harvested fruit, including ripe and part-coloured, was diseased. Seventeen days later, the incidence increased to 36 per cent. of the total sample, and on that occasion 44 per cent. of the ripe fruit, excluding part-coloured fruit, was affected.

Table 1.

Strawberry Fruit Losses From Gloeosporium sp.

Date.	Maturity.	No. Healthy.	No. Diseased.	Percentage Diseased.
28–10–52	Ripe and part-coloured	441	213	33
6–11–52	Ripe	615	491	44
	Part-coloured	264	0	0

Whole consignments of strawberry fruit have been condemned on arrival at the fresh fruit markets due to the appearance and spread within them of the ripe fruit rot. Invariably such losses have occurred when part-coloured fruit from diseased plantings have been consigned to interstate and intra-state markets.

During periods of wet weather, strawberry plantings which are established on poorly-drained soils are subject to the grey mould rot caused by a species of *Botrytis*. Fruit losses due to grey mould may occur throughout the harvesting period and such losses incurred during the late winter and early spring may be of greater economic significance than the later fruit wastage caused by the species of *Gloeosporium*.

IV. THE CAUSAL ORGANISM.

(1) Isolation.

Isolations were made from fruit lesions, stolons, leaf stalks and fruit stalks by tissue planting and conidial dilutions on to potato dextrose agar. Two isolates of the genus *Gloeosporium* which differed in morphology and cultural characters were obtained. One isolate, designated isolate *a*, was consistently obtained from all diseased tissues of the strawberry. The second isolate, isolate *b*, was obtained only on four occasions from diseased fruit and from leaf stalks. The relationship of this isolate to the disease is doubtful, and apart from a description of its morphological characteristics it receives only minor treatment in the discussion which follows.

(2) Morphology.

Strawberry isolate a.—Two types of mycelium are formed: (1) hyaline, thin-walled, septate, branched mycelium, 1–3 μ in diameter; and (2) dark-brown, thick-walled, branched mycelium with septations at short intervals, 6 μ in diameter. Conidia and chlamydospores are produced. Abundant conidia, which are unicellular, non-septate and cylindrical, develop directly from the mycelium. Oil drops are frequently formed within the conidia. Natural conidia vary in size, the range being 12.9–13 9 μ by 3.2–3.9 μ (range of conidial means). Non-setose acervuli form as a result of the compaction of mycelial strands. Masses of conidia, salmon-pink in mass, arise directly from the mycelium and appear as the acervulus erupts. Chlamydospores, which are irregular in shape and 4–6 μ in diameter, are produced as the dark-brown, thick-walled mycelium ages.

Strawberry isolate b.—The morphology of strawberry isolate *b* is similar to that of strawberry isolate *a*, except that conidia are formed in limited numbers and the size range is 7.0–8.8 μ by 2.3–2.8 μ (range of conidial means).

(3) Cultural Characters.

In strawberry isolate *a*, the aerial mycelium, in young colonies on potato dextrose agar, is white, compact and somewhat aggregated; in older colonies, a typical grey overgrowth develops. The substratum is pink-cream in young colonies, and scattered black areas with a dusky diffusion develop in older colonies. Pink spore masses influence the colour of the substratum.

(4) Pathogenicity Tests.

Tests using spore and mycelial inoculum demonstrated the pathogenicity of the two isolates. Pathogenicity ratings based on rate of lesion spread and mycelial penetration under identical conditions, using mycelial inoculum, showed that isolate *a* was more pathogenic than isolate *b*. The results of the strawberry inoculation are detailed in Table 2 and illustrated in Figs. 1 and 2.

Fig. 1.

Strawberry Fruit Inoculated in the Laboratory with *Gloeosporium* Isolate *a*. Appearance of the disease at different stages of lesion development.

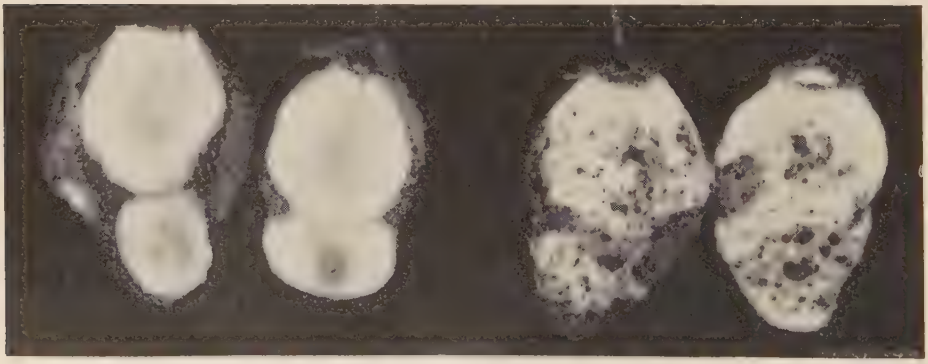

Fig. 2.

Laboratory Inoculation with *Gloeosporium* Isolate *a* (right) and Isolate *b* (left).

Table 2.

AVERAGE LESION DEVELOPMENT IN STRAWBERRIES INOCULATED WITH ISOLATES A AND B.

Maturity Group.	*Gloeosporium* Isolates.	Average Lesion Diameter (mm.) of 20 Fruit Inoculated.	
		4 days.	6 days.
Ripe fruit	*a*	6·2	15·0
	b	2·4	6·5
Green fruit (ripe in 3 days)	*a*	4·1	11·9
	b	1·7	3·6

(5) Cross-inoculation Studies.

For comparison with the strawberry organism two isolates of *Gloeosporium* from papaws naturally infected with ripe fruit rots were supplied by Mr. J. H. Simmonds, of the Science Branch. One of these, papaw isolate *a*, he considered to be morphologically similar to strawberry isolate *a*, while the other, papaw isolate *b*, was regarded as belonging to the *Glomerella cingulata* group.

(a) Strawberry Fruit Inoculations.

The strawberry and papaw isolates were tested for pathogenicity by inserting a P.D.A. culture into ripe fruit surface sterilized with 50 per cent. alcohol for 30–40 seconds. Papaw isolate *b* proved to be the most pathogenic isolate, while papaw isolate *a* and strawberry isolate *a* gave similar results in the cross-inoculation studies; these are summarised in Table 3.

Table 3.

RESULT OF INOCULATING GROUPS OF TWENTY STRAWBERRY FRUIT WITH FOUR GLOEOSPORIUM ISOLATES.

Gloeosporium Isolate.	Average Lesion Development.		Mycelial Growth Above Lesion.
	2 days.	5 days.	
Strawberry Isolate *a* ..	+	+++ ($\frac{1}{2}$)	Pinkish-white, 1–2 mm. elevated
Strawberry Isolate *b* ..	+	+++ ($\frac{1}{3}$)	Dense, white, 3–5 mm. elevated
Papaw Isolate *a* ..	+	+++ ($\frac{1}{2}$)	Pinkish-white, 1–2 mm. elevated
Papaw Isolate *b*	++	+++ (1)	Sparse, pinkish-grey, 0–$\frac{1}{2}$ mm. elevated

\+ White superficial mycelial *in situ* and advancing.
++ Lesion commencing to form.
+++ Typical sunken lesion with mycelial overgrowth.
(0–1) Approximate surface areas of fruit affected.

Re-isolation from the lesions confirmed the presence of the inoculated isolates.

(b) Papaw Fruit Inoculations.

Strawberry isolate *a*, papaw isolate *a* and papaw isolate *b* produced similar ripe fruit rot lesions when inoculated on a random basis into surface cuts on ripe papaw fruits. Strawberry isolate *b* was unable to form a typical lesion when inoculated into the same papaw fruits. The mycelium either remained dormant *in situ* or formed a small, dry, superficial lesion with little or no penetration. The results of the cross-inoculation into papaw fruits are summarised in Table 4. The inoculated organism was re-isolated.

Table 4.

Results of Inoculating Papaw Fruits with Four Gloeosporium Isolates.

Gloeosporium Isolate.	No. of Inoculation Sites.	Dormant Mycelium *in situ*.	Slight Penetration of Tissues.	Ripe Fruit Rot Lesion.
Strawberry Isolate *a*	46	1	0	45
Strawberry Isolate *b*	99	51	40	0
Papaw Isolate *a*	41	0	0	41
Papaw Isolate *b*	19	0	0	19

(c) Comparison of Spore Sizes.

Conidia of strawberry isolate *a*, papaw isolate *a* and papaw isolate *b*, which developed on three different sporulation substrates (P.D.A. and inoculated strawberry and papaw fruit), were used. Five groups of 30 spores were measured in each case; the range of means and overall mean values are summarised in Table 5.

Table 5.

Summary of Spore Measurements (μ).

Gloeosporium Isolate.	Length.			Width.		
	P. D. A.	Strawberry.	Papaw.	P. D. A.	Strawberry.	Papaw.
Strawberry Isolate *a* ..	(10·2–11·8) 10·8	(10·9–11·5) 11·2	(10·5–13·2) 11·9	(2·8–3·3) 3·1	(3·4–3·7) 3·5	(2·8–3·4) 3·2
Papaw Isolate *a*	(9·9–10·9) 10·5	(10·2–12·4) 11·1	(9·8–12·1) 10·9	(2·8–3·2) 3·0	(3·5–3·7) 3·6	(3·0–3·4) 3·2
Papaw Isolate *b*	(13·1–14·8) 13·9	(12·7–15·7) 14·1	(14·2–15·5) 14·7	(3·3–3·4) 3·3	(3·8–4·5) 4·2	(4·0–4·3) 4·1

The mean values were analysed statistically to determine the significance of differences between the three isolates. There were no significant differences in length or width of strawberry isolate *a* and papaw isolate *a* on the same medium and overall media. Papaw isolate *b* significantly exceeded the other two isolates in length and width at the 1 per cent. level.

As a result of the cross-inoculation studies with four isolates of *Gloeosporium* and the comparison of spore measurements, it is concluded that strawberry isolate *a* and papaw isolate *a* are identical organisms and cause ripe fruit rots of the strawberry and papaw.

(6) Taxonomy.

Frequent examinations of naturally infected strawberry tissues did not reveal the perfect stage of the causal organism. It was not formed in laboratory culture nor was it induced by applying the technique described by Chona and Srivastava (1952).

In view of the results of the genetic studies of Chilton and Wheeler (1949), it is conceivable that the perfect stage of the strawberry isolates does not exist in nature. They found that conidial strains of *Glomerella cingulata* which do not possess genes or gene factors necessary for the development of the perithecial stage arise in culture as a result of gene mutations. A similar condition may arise in nature with the species of *Gloeosporium* causing the ripe fruit rot of the strawberry.

In the absence of the perfect stage it is not considered desirable to assign a binomial to the conidial stage without further investigating possible relationships with other species belonging to the form genus *Gloeosporium*.

(7) Latent Infection.

Circumstantial evidence noted throughout the strawberry ripe fruit rot investigations strongly suggested a mechanism for latent infection by spores of the strawberry organism. A latent infection in various fruit rot organisms has been postulated by a number of workers, while Simmonds (1940) illustrated the mechanism involved in the case of *Gloeosporium* spp. on banana, mango and papaw.

Following the technique developed by Simmonds, three groups of 30 green, fully developed fruit were surface-sterilized and treated as follows:— (1) incubated at 25·5 deg. C.; (2) immersed in a conidial suspension of strawberry isolate *a*, then one hour later surface-sterilized with 1/1,000 mercuric chloride; and (3) immersed in a conidial suspension, then incubated for 27

Table 6.

Latent Infection Studies with Strawberry Fruit.

Group.	Healthy Fruit.	Ripe Fruit Rot (*Gloeosporium* sp.)
Uninoculated; surface sterilized	29 (1 *Botrytis* sp.)	0
Conidial suspension; surface sterilization	29	1
Conidial suspension; 27 hours' incubation; surface sterilization	10	20

Fig. 3.

Six Days' Growth of Strawberry Isolate *a* on P.D.A. Slopes at a Series of Temperatures (°C.).

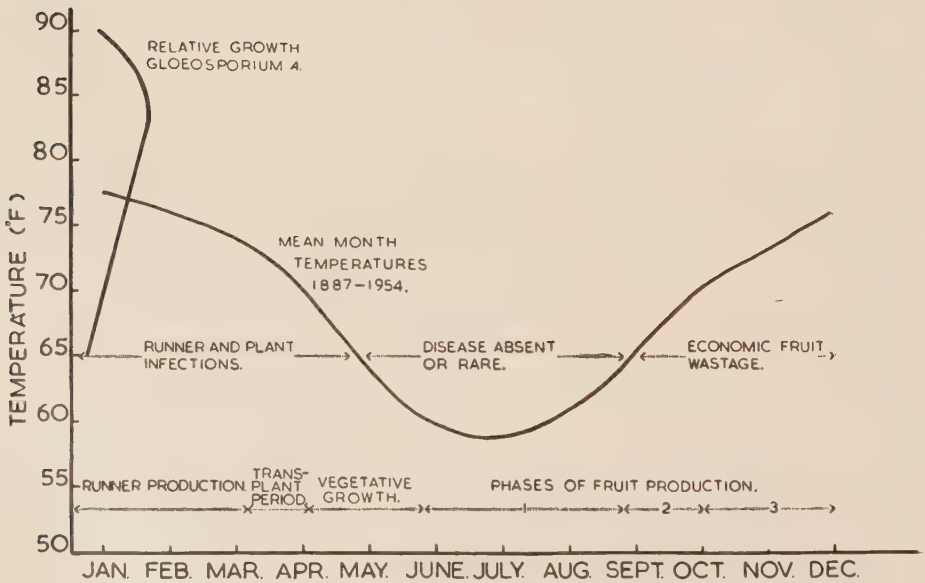

Fig. 4.

Correlation of Strawberry Ripe Fruit Rot Incidence with Seasonal Fruit Production and Temperature.

hours at 25·5 deg. C., then sterilized by mercuric chloride and further incubated at 25·5 deg. C. The fruit were examined when ripe five days later. The results, which are summarised in Table 6, suggest that spores are able to penetrate the epidermis of the green fruit within 27 hours after inoculation and form typical lesions when the fruit are ripening.

(8) Temperature Studies.

Single-spore colonies of the two strawberry isolates were cultured in small, flat-sided bottles containing 15 ml. P.D.A. The bottles were incubated in a multiple temperature incubator at 13 different temperatures from 14 deg. to 33·5 deg. C., and growth measurements were recorded daily for seven days. Over this temperature range the growth-temperature curves for each isolate were similar, although the growth rate of strawberry isolate *a* exceeded that of strawberry isolate *b*. Mycelial growth steadily increased with increasing temperature from 14·0 deg. C. (57·2 deg. F.) to the optimum temperature of 28·0 deg. C. (82·4 deg. F.), and beyond that temperature mycelial growth rapidly decreased with increasing temperature. The growth-temperature relationships of strawberry isolate *a* are illustrated in Figs. 3 and 4.

P.D.A. cultures of strawberry isolate *a* were able to withstand temperatures below freezing point. Spores and mycelium remained viable when exposed to —35 deg. C. for three days. When heated for 10 minutes in a constant temperature water-bath, strawberry isolate *a* was shown to have an upper thermal death point between 50 deg. and 60 deg. C.

Groups of 10 ripe strawberry fruit surface-sterilized in 50 per cent. alcohol were immersed in a conidial suspension of strawberry isolate *a* and incubated at different temperatures. At a temperature of 27·8 deg. C., which approximates the optimum temperature for mycelial growth, strawberry isolate *a* initiated a ripe fruit rot lesion in two days and complete mycelial penetration followed in a further 2–3 days. Table 7 details the results for lesion initiation and mycelial penetration of ripe fruit when incubated at temperatures ranging from 15·5 deg. to 27·8 deg. C.

Table 7.

Period Required for Lesion Initiation and Complete Mycelial Penetration of Strawberry Fruit at Various Temperatures.

Temperature. °C.	(°F.)	Lesion Initiation (days).	Complete Mycelial Penetration (days).
15·5	(59·9)	7	11–14*
17·0	(62·6)	6	9–11
18·6	(65·5)	5	9–10
21·2	(70·1)	4	6–7
24·8	(76·6)	3	4–6
27·8	(82·0)	2	4–5

* Range indicates time taken from first to last fruit in the groups of 10.

V. FIELD CONDITIONS INFLUENCING THE SEASONAL OCCURRENCE OF THE DISEASE.

The absence of the strawberry ripe fruit rot each year during the winter period of fruit harvest and the outbreaks of the disease which followed the appearance of localised traces of infection during the early spring directed attention towards the prevailing climatic conditions. The fluctuating order of disease incidence from the spring until the end of summer suggested that the increasing seasonal temperatures were an important factor conditioning the development of the disease.

Twelve estimates of fruit losses which were made in field plantings during the years 1952 to 1954, together with associated meteorological data, are summarised in Table 8. The mean daily temperatures are averaged for the week preceding each estimate of the disease, and the amount of rain and number of wet days for the fortnight preceding each estimate are given. The records of the disease were taken from diseased strawberry plantings located within a radius of 20 miles of the Brisbane Meteorological Station.

From Table 8, a mean daily temperature of 65 deg. F. (18·3 deg. C.) was selected as the critical temperature above which economic levels of fruit wastage (greater than 10 per cent. loss) may be expected to occur. In Table 9, the mean monthly temperature for 1951–1954 shows that the months of June, July and August experience temperatures below the arbitrary level of 65 deg. F., and apart from the last week or two in August, the disease has not been observed during that period of early fruit production. Figure 4 summarises the relationship of ripe fruit losses to temperature.

Table 8.

Strawberry Ripe Fruit Rot Estimates and Meteorological Data.

Date.	Fortnightly Total.		Weekly Average Mean Daily Temp. (°F.).	Fruit Rot. (%)	District.
	Rainfall (in.).	Wet days.			
24–8–54	4·10	5	63	Trace	Sunnybank Wellington Point
1–9–53	3·50	5	62	3	Mitchelton
10–9–53	3·70	5	64	Trace	Ormiston
4–10–54	1·30	7	65	60	Manly
22–10–52	1·10	5	68	77	Ormiston
28–10–52	1·10	6	68	33	Ormiston
6–11–52	·80	5	79	44	Ormiston
11–11–54	3·00	8	71	>50	Brisbane
1–12–53	2·20	4	76	>50	Brisbane
7–12–53	2·20	4	75	20	Mount Gravatt
21–12–54	1·20	5	74	>50	Sunnybank
22–12–52	·90	4	74	Trace	Ormiston

Table 9.

MEAN MONTHLY TEMPERATURES (°F.)—BRISBANE (1951–1954.)

Month.	1951.	1952.	1953.	1954.	1887–1954.
January	73	79	74	74	77
February	75	76	75	76	76
March	74	74	73	77	74
April	68	71	72	71	70
May	62	64	65	65	64
June	61	61	60	60	60
July	58	60	59	62	59
August	59	62	60	61	61
September	64	65	65	64	65
October	69	69	70	68	70
November	74	75	74	73	73
December	76	76	78	76	76

Another field condition affecting the strawberry microclimate during the susceptible period of the year is plant humidity, which is influenced by the amount and duration of rainfall, dews and the prevailing winds. Although with few exceptions overhead irrigation is applied to strawberry plantings in the districts surrounding Brisbane, serious outbreaks of the disease tend to follow rainfall which extends over several days. Given suitable temperature conditions, rainfall appears to be associated with the outbreaks of strawberry ripe fruit rot, but rainfall alone is clearly insufficient.

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BORON AND MOLYBDENUM REQUIREMENTS OF THE TOMATO CROP IN THE GRANITE SOILS OF THE STANTHORPE DISTRICT.

By T. J. BOWEN, B.Sc. (Formerly Horticulturist, Horticulture Branch).

SUMMARY.

Increased yields of tomatoes were obtained from boron (borax applied both as a soil dressing and as a foliage spray), from molybdenum (ammonium molybdate applied as a foliage spray), and from molybdenum plus boron. The increases were of the same magnitude in all three treatments.

No additive effects were noted in plants receiving both molybdenum and boron. It is postulated that a deficiency of some other element restricted the yield potential of the crop and masked the additive effect expected from the combined boron-molybdenum treatments.

I. INTRODUCTION.

The Granite Belt, an elevated plateau some 2,000–3,000 ft. high in south-eastern Queensland, is an important source of supply of tomatoes, beans and other small crops during the summer months. The soils, which are derived from granite, are typically infertile, are not always well drained, and tend to become highly acid when continuously cropped. Complete fertilizers containing nitrogen, phosphorus and potassium are therefore essential for normal production. Trace element deficiencies, though common in pome fruits (Ward 1946), have only recently attracted attention in vegetable crops in this district.

This may be due partly to the fact that, in the absence of irrigation facilities, production is somewhat speculative and crop failures are indiscriminately attributed to soil moisture deficiencies or excesses, or alternatively to the inroads of disease. In any case, gross abnormalities in plant growth such as those associated with deficiencies of boron in beetroot and cauliflower are virtually unknown, as these crops are not grown on a commercial scale. However, symptoms of molybdenum deficiency have been recorded in some plantings of beans and cucumbers.

The available soil analytical data suggested that boron and molybdenum were, at best, present only in marginal amounts and that a response might be obtained to treatment in crops such as tomato, which do not exhibit conspicuous reactions to soil deficiencies of these trace elements.

Accordingly, trials were established in 1953-54 at Bapaume and in 1954-55 at Amiens to determine, among other things, the effect of molybdenum, boron, and molybdenum plus boron on plant growth and yield in the tomato crop.

II. 1953-54 TRIAL.

(1) Treatments.

A. *FTE**.—Applied in the drill two weeks before planting at the rate of 1 cwt. per acre.

B. *Boron.*—Borax applied as a soil dressing in the drill one week before planting at the rate of 15 lb. per acre.

C. *Boron plus molybdenum.*—Borax applied as a soil dressing in the drill one week before planting; ammonium molybdate (1/1,600) applied as a foliage spray two weeks after transplanting.

D. *Molybdenum.*—Ammonium molybdate (1/1,600) applied as a foliage spray two weeks after transplanting.

E. *Urea*†.—Applied as a 1 per cent. foliage spray shortly after fruit set in the first hand and again two weeks later.

F. Control.

(2) Procedure.

The experiment was laid down as a 6 x 4 randomised block with eight plant plots, the plants being established on the square at a 6 ft. x 6 ft. spacing.

Seed of the variety Q2 (a certified strain of Grosse Lisse) was sown on Nov. 11 and the seedlings were transplanted into the field on Dec. 28. Fertilizer schedules were those normally used in the district, viz., basal 5:13:5 at 8 cwt. per acre; side 5:13:5 at 2 cwt. per acre.

The crop was grown without irrigation and encountered dry conditions during the early stages. Heavy rain fell in February (Table 1) and from then on until the end of the harvesting period soil moisture was more than adequate for plant development.

Table 1.

RAINFALL AT STANTHORPE.

Month.	1953–54.	1954–55.
	in.	in.
December	·46	3·22
January	1·24	·98
February	11·93	2·43
March	1·15	3·11
April	·91	2·56
Total	15·69	12·30

* FTE is a commercial preparation of ground glass containing 10 per cent. ferric oxide, 2 per cent. boric oxide, 4 per cent. zinc oxide, 4 per cent. cuprous oxide, 4 per cent. manganese oxide and 0.2 per cent molybdenum trioxide.

† The urea treatment was included as a matter of convenience to assess the effect of supplementary nitrogen applied as a foliage spray on plant growth and yields.

Flowering commenced on Jan. 25 and the first pick was made on Mar. 3. Harvesting continued until Apr. 15. All fruit exceeding 2 in. in diameter was classed as marketable.

(3) Results.

The results of the 1953-54 trial are recorded in Table 2.

Table 2.

EFFECT OF TREATMENT ON YIELDS OF MARKETABLE FRUIT (1953–54).

Treatment.	Yield.		Significantly Exceeds at.	
	Lb./Plot.	Equiv. Cases/ac.	5% Level.	1% Level.
D. Mo	202·2	1,273	A	F
C. Bo + Mo	199·5	1,257	F	
B. Bo	188·6	1,189	F	
E. Urea	186·0	1,172		
A. F. T. E.	171·5	1,081		
F. Control	158·2	997		

III. 1954-55 TRIAL.

Although responses were obtained from molybdenum, boron, and boron plus molybdenum in 1953-54, no significant differences between the three treatment schedules were recorded. A somewhat similar trial was therefore carried out in 1954-55, an additional treatment in this case being the trace element cobalt.

(1) Treatments.

A. Control.

B. *Molybdenum.*—Ammonium molybdate (1/1,600) foliage spray applied two weeks after transplanting

C. *Molybdenum.*—Ammonium molybdate (1/1,600) foliage spray applied two weeks after transplanting and again two weeks later.

D. *Boron.*—Borax (1/250) foliage spray applied two weeks after transplanting.

E. *Boron plus molybdenum.*—Ammonium molybdate plus borax (1/6/1,600) foliage spray applied two weeks after transplanting.

F. *Cobalt.*—Cobalt chloride (1/1,600) foliage spray applied two weeks after transplanting.

(2) Procedure.

The experimental layout and fertilizer schedules were identical with those of the 1953-54 trial. The crop was, however, planted a little earlier—on Dec. 30—and harvesting began on Feb. 24, as against Mar. 3 in 1954. Growing conditions were relatively good during the growing period (Table 1).

(3) Results.

The results are presented in Table 3.

Table 3.

EFFECT OF TREATMENT ON YIELDS OF MARKETABLE FRUIT (1954–55).

Treatment.	No. of Fruit.	Significantly Exceeds at 1% level.	Yield.		
			Lb./ac.	Equiv. cases/ac.	Significantly Exceeds at 1% level.
B. Mo (single)	900	F, A	238·0	1,499	F, A
D. Bo (single)	889	F, A	234·2	1,475	F, A
E. Mo + Bo (single) ..	854	F, A	232·2	1,462	F, A
C. Mo (double)	828	A	225·2	1,418	F, A
F. Co (single)	690		184·8	1,164	
A. Control	602		168·2	1,060	

IV. DISCUSSION.

(1) General.

In these two trials, responses were obtained from boron, molybdenum and boron plus molybdenum. Mean yields per plot from these treatments in each year were as follows:—

1953–54—Mo (single spray), 202·2 lb.; Mo plus Bo (soil dressing), 199·5 lb.; Bo (soil dressing), 188·6 lb.; control, 158·6 lb.

1954-55—Mo (single spray), 238·0 lb.; Bo (spray), 234 lb.; Mo plus Bo (spray), 232·2 lb.; Mo (double spray), 225·2 lb.; control, 168·2 lb.

The overall higher yields in 1954–55 can be ascribed to the better growing conditions during the early stages of plant development.

The results from both trials, however, indicate that:—

(1.) Even in the absence of typical symptoms of deficiencies in boron and molybdenum, treatment with these trace elements increased production in the tomato crop.

(2.) A response to boron occurred in the absence of additional molybdenum and a response to molybdenum in the absence of additional boron. There were, however, no additive effects when both were applied to the crop.

(3.) The response to a single application of molybdenum was comparable to that obtained with two sprays applied at an interval of two weeks. The second treatment was therefore redundant.

(2) Status of Boron in the Soil.

Disorders in deciduous fruit trees due to boron deficiency have been recognized for many years in the Granite Belt and corrective treatments are an essential feature of efficient orchard management. For practical purposes, it is assumed that when the boron content of the soil falls below 0·3 p.p.m. additional

boron is probably required. Even at these levels tree reaction varies from season to season in pome fruits according to the amount of rain which falls prior to and during the growing period. In the soil used for these trials, the boron level was 0·3 p.p.m. (soluble boron in hot water extract—determined by Agricultural Chemist).

The experimental data presented above suggest that the cropping potential of the tomato crop is substantially reduced by lack of sufficient boron in the soil. Periodic applications of borax therefore seem desirable in areas where vegetables are regularly grown.

(3) Status of Molybdenum in the Soil.

Although molybdenum deficiencies have long been recognized in coastal areas of southern Queensland, where the more susceptible crops such as cauliflower and rockmelon are grown extensively (Morgan and Henderson 1950), the economic significance of possible deficiencies at Stanthorpe were not realised until 1952, when spot applications of an ammonium molybdate spray to beans and some other crops substantially improved leaf colour and plant growth. The shortage of available molybdenum may, in part, have been aggravated by the progressively increasing acidity of the soil following the use of acidic fertilizers at heavy rates during and subsequent to the Second World War.

The problem at Stanthorpe, however, is not so much the correction of the disorder when molybdenum deficiency symptoms develop as the desirability of treatment at an earlier date; production from commercial crops may appear satisfactory and yet be far below the potential of the land when supplies of molybdenum are increased (Hewitt and Jones 1952).

(4) Additive Effects of Boron and Molybdenum.

Deficiencies of boron and molybdenum have distinctive effects on plant metabolism.

Adequate amounts of boron are essential for cell multiplication in the cambial tissues and a deficiency is followed by abnormalities in the associated parts of the plant.

A deficiency of molybdenum, on the other hand, causes an upset in nitrogen utilization, particularly when the crop is grown on highly acidic soils. Typical symptoms are chlorosis, stunting and malformation of the lamina and necrotic lesions along the margin of the leaves.

Where both boron and molybdenum are deficient in the soil and responses are obtained from corrective measures for each, one would expect a greater response when both are applied to the crop. No such additive effect was recorded in the trials. This could have been due to target spot infection which reduced

the cropping period and total yields from which significant differences were determined or, alternatively, to deficiencies of other essential nutrients such as nitrogen. The latter seems the more probable in view of the response to a urea spray in the 1953-54 trial.

(5) Treatment Schedules.

Reserves of both boron and molybdenum in many soils are limited and deficiencies are therefore most pronounced in land which has been extensively farmed for long periods. Such conditions exist in the older established horticultural areas, such as the Brisbane district.

In this and similar areas, commercial crops are good indicator plants for boron, and it is standard practice to use a pre-planting dressing of borax on beetroot and cauliflowers. Boron residues from these crops appear adequate for tomatoes, beans, cabbage and other plants in the rotation. At Stanthorpe, where few beetroot and cauliflower are grown, growers have no ready guide to the need or otherwise of boron applications in commercial crops.

The present trials reveal a deficiency of boron both on virgin ground (1954-55) and on land which has been cultivated for some time (1953-54). It seems essential therefore that, in the absence of indicator plants in the rotation, boron should be applied either as a soil dressing of borax or in a foliage spray containing borax every second or third year on land which is continually cropped. This practice may not provide optimum amounts of boron but it should increase yields without any risk of residues accumulating in the soil in toxic quantities.

The molybdenum position is much the same as that for boron, and regular treatments appear to be necessary for optimum yields. However, the risk of toxic residues building up from foliage sprays of ammonium molybdate applied to each crop is negligible. For the time being, therefore, treatment of all crops at an early stage in growth is considered desirable.

V. CONCLUSIONS.

In trials carried out in the Stanthorpe district, increased yields of tomatoes were obtained following application of boron as borax, molybdenum as ammonium molybdate, and boron plus molybdenum. The second of these trials was established on virgin ground and it is therefore postulated that reserves of these elements on most farms are below requirements for maximum production.

Gross abnormalities in plant growth due to deficiencies of boron and molybdenum have not been recorded in the tomato crop at Stanthorpe. Indicator plants for deficiencies of these elements are not widely grown commercially in

the district. The practice adopted elsewhere of applying corrective measures to susceptible crops where they occur in the rotation is therefore hardly practicable.

At Stanthorpe, the best procedure may be to apply a soil dressing of borax at least every second year to land which is regularly cropped to vegetables and to apply an ammonium molybdate spray to all crops.

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STUDIES OF THE COCCOIDEA. 6. NEW GENERA AND NEW SPECIES OF ASPIDIOTINI.

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SUMMARY.

New species of Aspidiotini with a constricted thorax and dorsal pygidial chitinization are Mimeraspis cuspilobis, M. rotundus, Myrtophila curvata, M. adnatae, M. suticollis, Dichosoma convexa, Achorophora obliqua, A. divergens, Diaphoraspis orbata, D. incisa, D. compacta, Aspidonymus woodwardi, Acontonidia triangularis, Duplaspidiotus magnus **and** Pseudotargionia inconspicua. **The first seven of the nine genera mentioned are also new.**

INTRODUCTION.

Among the scale insects described by previous authors are a few species such as *Neomorgania eucalypti* (Mask.) in the Aspidiotini having a deeply constricted thorax and a heavily chitinized body, particularly on the pygidium. The dorsal pygidial chitinization is typically reticulate but is sometimes stipple-like or with widely separated and irregularly branched sutures. Other species with these general characters have been described by the present author (Brimblecombe 1953, 1956), and 15 species dealt with in this paper also belong to the group. It appears therefore that species of this kind are numerous in Australia and represent a dominant part of the Aspidiotine fauna in this country.

MIMERASPIS new genus.

Genotype—Mimeraspis cuspilobis n.sp.

Characters.—Adult female with a deep thoracic constriction, anterior portion dome-shaped, posterior portion semicircular. Pygidium with median lobes contiguous and united basally, with a median dorsal groove, other lobes if present small elongate points. Basal scleroses absent. Paraphyses in the first and second interlobal areas. Plates present in the first, second and third interlobal spaces, simple or variously branched. Dorsal ducts in series on abdominal segments and present on the metathorax. Perivulvar pores absent, anal opening small. Pygidium dorsally with a reticulate chitinization. Anterior spiracles with associated but dispersed pores.

Notes.—The genus *Mimeraspis* resembles *Neomorgania* and *Pseudotargionia* in having a thoracic constriction, prominent median lobes, a small anal opening close to the lobes, pores associated with the anterior spiracles and dorsal reticulated chitinization on the pygidium. The ducts, duct orifices and dispersed parastigmatic pores differ from those of *Neomorgania.* The contiguous median lobes distinguish the genus from *Pseudotargionia.*

Mimeraspis cuspilobis n.sp.

(Figs. 1 and 2.)

Localities and host.—Queensland: Type and paratype from Boonah on *Callistemon viminalis* (Soland. ex Gaertn.) G. Don ex Loud., July 1954; other paratypes from Forest Hill on *Callistemon viminalis,* Jan. 1953.

Fig. 1.

Mimeraspis cuspilobis. Outline of body (160X).

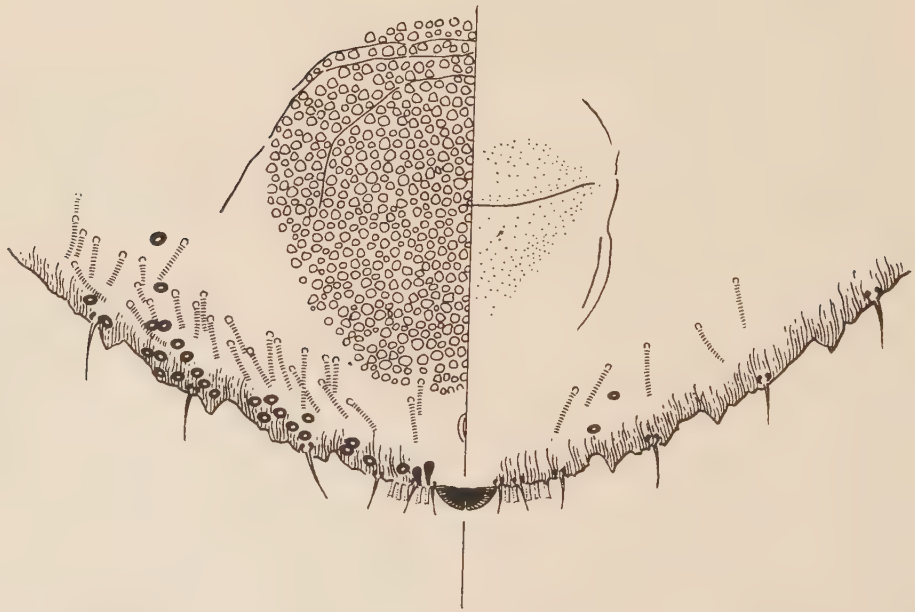

Fig. 2.

Mimeraspis cuspilobis. Enlargement of pygidium (300X).

Habit.—Insects sparse on foliage. Scale circular, 0.8 mm. diam., dark fawn in colour, margin paler, the dark orange first pellicle surrounded by a greyish band.

Recognition characters.—Adult female with a deep thoracic constriction, anterior portion dome-shaped, with a lateral short stout spine and lateral margins posteriorly convergent, posterior portion wider, semicircular, with segmental marginal points. Length of slide specimen 0.6 mm., width 0.5 mm. Pygidium not differentiated, apex broadly rounded. Median pair of lobes only, contiguous, as wide as long, united basally, a division apparent dorsally, subtriangular but the outer margin curved, median portion more strongly chitinized. Basal scleroses absent. Paraphyses as a pair of chitinized thickenings in the first and second interlobal areas. Plates shorter than the lobes, slender, simple or slightly branched, two in the first interlobal space, three in the second, one in the third. Spines longer than the lobes. Dorsal ducts submarginal, slender, length mostly twice the distance from the bases of the lobes to the anal opening; orifices marginal and submarginal on abdominal segments and metathorax, mostly in segmental series. Perivulvar pores absent. Anal opening small, one to one-and-a-half times the lobe's length from the bases of the lobes. Abdominal margin with chitinized triangular points on segments 3, 4 and 5. Median pygidial area with a fine reticulated chitinization, body elsewhere may have a fingerprint chitinization or a dense uniform chitinization. Anterior spiracles with five to seven associated but widely dispersed pores.

Notes.—All specimens do not possess the thoracic lateral spine. On the more chitinized specimens the pores on the metathorax are not obvious. The pygidial margin may have a densely chitinized rim. A slight thickening near the base of each lobe, actually between the lobe and the associated spine, may give the appearance of three paraphyses in the first interlobal area. Sometimes the median lobes are so close as to appear united except for a medium dorsal groove. The paraphyses in the second interlobal area may be indistinct when the body margin is heavily chitinized. Ventral chitinization on the pygidium may be like a stippling. Posterior spiracle may have one associated pore.

Type Reg. No. T.5608 and paratypes Nos. T.5609 and T.5610 in the Queensland Museum.

Mimeraspis rotundis n.sp.

(Figs. 3 and 4.)

Type locality and host.—South Australia: Mann Range, on *Melaleuca dissitiflora* F. Muell., Aug. 1954 (J. B. Cleland per S. T. Blake).

Habit.—Insects thinly scattered on leaves. Scale convex, circular, 1·0 mm. diameter, light fawn in colour; the apical suffusion is often rubbed off, revealing the orange brown to dark brown pellicles.

Recognition characters.—Adult female subcircular, with a deep thoracic constriction; anterior portion dome-shaped, about twice as wide as long, lateral margins almost straight and strongly convergent posteriorly; posterior portion wider, semicircular. Length of slide specimen 0.6 mm., width 0.5 mm. Pygidium comparatively large; apex broadly rounded. Three pairs of lobes. Median lobes slightly longer than wide, contiguous, inner margins almost straight, outer margins convergently curved, yoked and densified basally. Second pair of lobes elongate points, one third the length of the median lobes, separated from the median lobes by half of a median lobe's width. Third pair of lobes similar to the second, and separated by the width of a median lobe. Basal scleroses absent. Paraphyses

Fig. 3.

Mimeraspis rotundus. Outline of body (150X).

Fig. 4.

Mimeraspis rotundus. Enlargement of pygidium (290X).

as an uneven pair in the first and second interlobal areas, all anteriorly enlarged, the innermost in the first interlobal area shorter than the median lobes, the outer paraphysis of the first interlobal area and the inner of the second subequal to half the length of the innermost paraphysis. Plates shorter than the median lobes, two in the first interlobal space, three in the second and one in the third, with various apices from simple to three or four branched. Spines adjacent to the median lobes shorter than the lobes, other abdominal spines longer. Dorsal ducts short, orifices with chitinized rims, in segmental series with shorter intervening series, two in the first interlobal area, three or more in the second and 10 or more in the third, slightly more in number in anterior series, with a series on the metathorax. Perivulvar pores absent. Anal opening small in a narrow inverted V, slightly more than the length of the median lobes from the bases of these lobes. Vulva large, curved. Dorsal chitinization as a fine reticulation and ventrally like a stippling. Anterior spiracles with three or four dispersed associated pores. Posterior spiracle without associated pores.

Notes.—A marginal concavity may be present posteriorly to the lateral extremities of the anterior body portion. Sometimes only two pores are associated with the anterior spiracles. Some duct orifices are marginal and the numbers in the series may vary from those given above, since there are usually more duct heads evident than recorded orifices. The median lobes sometimes are so close as to appear united. The body of mature specimens may be completely heavily chitinized. This species differs from *M. cuspilobis* in having three pairs of lobes, the parastigmatic pores are fewer in number and are not so widely dispersed.

Other material examined is from Western Australia, Mt. Magnet, on *Eucalyptus* sp., possibly *E. spathulata* Hook., Jan. 1916 (Clarke).

Type Reg. No. T. 5611 and paratypes Nos. T. 5612 to T. 5614 in the Queensland Museum.

Myrtophila new genus.

Genotype.—Myrtophila curvata n.sp.

Characters.—Adult female with a deep thoracic constriction, anterior portion dome-shaped, posterior portion semicircular. Pygidium with three pairs of lobes, second and third pairs may be reduced to small points. Basal scleroses absent. Paraphyses as short yoked thickenings in the first and second interlobal areas. Plates present in the median, first and second interlobal spaces, slender in the median space and simple or branched in the first and second spaces. Dorsal duct heads large in the median area, progressively smaller in the first, second and other duct furrows. Perivulvar pores absent. Anal opening small. Pygidium with a dorsal reticulate chitinization. Anterior spiracles with associated pores.

Notes.—The genus *Myrtophila* is related to *Pseudotargionia, Neomorgania* and *Mimeraspis.* The independent median lobes and intervening plates are characters separating it from *Neomorgania* and *Mimeraspis,* while the large dorsal ducts in the median area and the short uniform paraphyses distinguish it from *Pseudotargionia.*

Myrtophila curvata n.sp.

(Figs. 5 and 6.)

Localities and hosts.—Queensland: Type and paratype from Tugun on *Leptospermum stellatum* Cav., Jan. 1950; other paratypes from Eprapah, near Cleveland, on *Leptospermum flavescens* Sm., Sept. 1954.

Habit.—Insects single and sparse, mostly in leaf axils. Scale circular, 0·75 mm. diameter; brown to dark brown, margin fawn; first pellicle dark brown.

Recognition characters.—Adult female with a deep thoracic constriction; anterior portion dome-shaped with a short stout lateral spine, lateral margins posteriorly convergent; posterior portion wider, semicircular. Length of slide specimen 0.6 mm., width 0.55 mm. Pygidium not differentiated, apex very broadly rounded. Three pairs of lobes, median normal, second and third pairs tooth-like with further chitinized marginal serrations. Median pair of lobes wider than long, slightly convergent, separated, apex broadly curved but with four indentations. Second pair of lobes nearly as wide as the median, wider than long, triangular, apex acute, separated from the median by half to three-quarters of the latter's width. Third pair of lobes similar to the second, but smaller, separated from the second by half its own width. Basal scleroses absent. Paraphyses as short paired chitinous thickenings in the first and second interlobal areas, shorter than the median lobes. Two plates in each of the median first and second interlobal spaces, as long as the lobes, slender in the median space, broad and fimbriate in the first and second interlobal spaces. Spines near lobes as long as the median lobes, others longer and hair-like. Dorsal ducts of different sizes, wider medianly where orifices are marginal, length in this area reaching almost to the anal opening; one or two wide ducts in the median interlobal area, two in the first duct furrow, four in the second furrow, with the size progressively smaller internally and anteriorly,

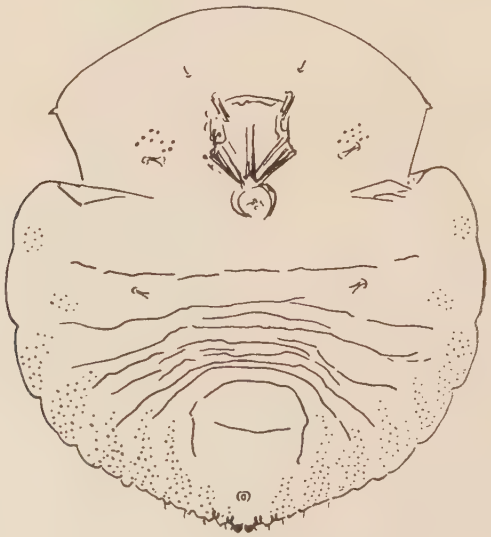

Fig. 5.

Myrtophila curvata. Outline of body (100X).

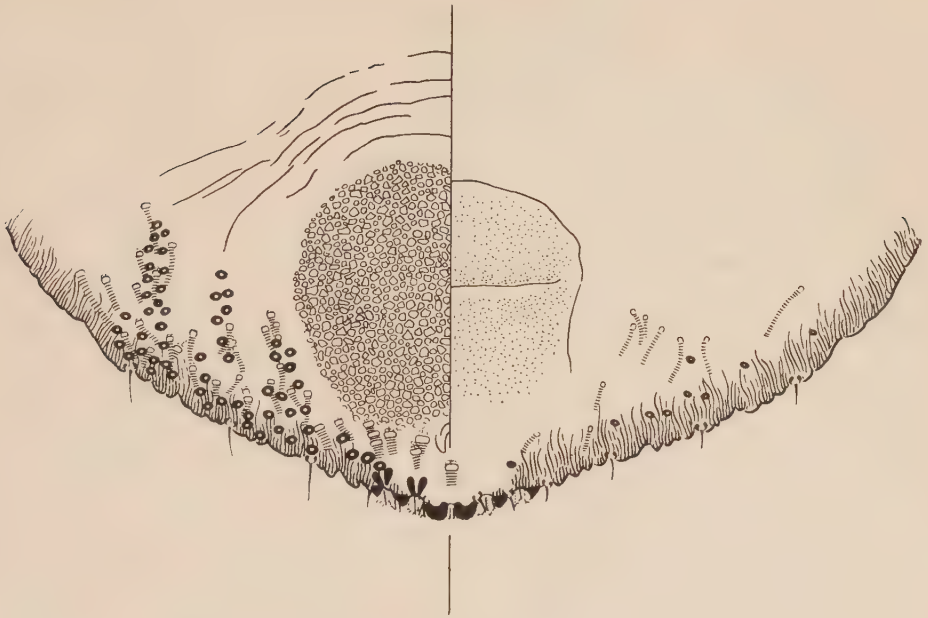

Fig. 6.

Myrtophila curvata. Enlargement of pygidium (240X).

about 12 ducts in the third furrow, and more in anterior abdominal segments. Anal opening small in an inverted V, three times the median lobe's length from the bases of the lobes. Perivulvar pores absent. A central dorsal chitinous reticulation on the pygidium, body surface elsewhere with a light finger-print chitinization. Anterior spiracles with seven to nine associated pores.

Notes.—Plates in the median interlobal space are not always discernible. The second and third lobes may appear to be chitinous serrations widely different in shape from the median lobes. In the related species *M. adnatae* n.sp. there are no obvious second and third lobes yet normal lobes in these positions occur in *M. suticollis* n.sp. The yoked paraphyses may appear to be chitinized portions of a thickened marginal rim. About nine apparently small duct orifices, represented by clear spots in the chitinized derm, occur sub-marginally on the metathorax and mesothorax. Duct orifices generally may have chitinized rims and some appear to be on an angle to the median line. The median lobes may be much wider than those of the second pair. Sometimes the lateral thoracic spur is absent. With heavy chitinization the median lobes tend to lose the indentations and the apices, especially of the second and third pairs, become rounded. Two wide ducts may be present in the median interlobal area. Body of old specimens may become broader, and as much as twice as wide as long. Posterior marginal thickenings may be present ventrally on pre-pygidial segments.

Type Reg. No. T. 5615 and paratypes Nos. T. 5616 to T. 5618 in the Queensland Museum.

Myrtophila adnatae n.sp.

(Figs. 7 and 8.)

Locality and host.—Queensland: Yelarbon on *Melaleuca adnata* Turez, Oct. 1954.

Habit.—Insects mostly single on the basal half of the upper surface of the rather small leaves. Scale circular, 0·8 mm. in diameter, white to light fawn in colour, pellicles almost black.

Recognition characters.—Adult female with a deep thoracic constriction; anterior portion dome-shaped, lateral margins posteriorly convergent; posterior portion wider, semi-circular. Length of slide specimen 0.6 mm., width 0.5 mm. Pygidium not differentiated, comparatively large, apex very broadly rounded. Median lobes slightly longer than wide, inner margin slightly concave, outer margin broadly curved to a subapical indentation, apex rounded, separated by one-half to two-thirds of a lobe's width. Second and third pairs of lobes apparently absent, intervening interlobal spaces wide. Basal scleroses absent. Paraphyses in the first and second interlobal areas as pairs of short chitinous thickenings. Plates present in the median first and second interlobal spaces; two in the median, slender apex truncate, as long as the lobes, two in the first space, longer than the lobes and fimbriate, two in the second space similar to those in the first. Spines adjacent to the median lobes as long as the lobes, other spines longer. Dorsal ducts short, of different sizes; a broad duct in the median interlobal area, reaching to the anal opening, two in the first interlobal area and one wide and one small in the second; ducts smaller away from the margin and anteriorly; orifices mostly close to the margin, a few submarginally on the metathorax and mesothorax. Perivulvar pores absent. Anal opening in an inverted V, twice a lobe's length from the bases of the lobes. Vulva wide. Lateral thoracic spur absent. Pygidium with a dorsal reticulate chitinization. Scalloped or palisade chitinization ventrally on pre-pygidial abdominal segments. Anterior spiracles with three associated pores.

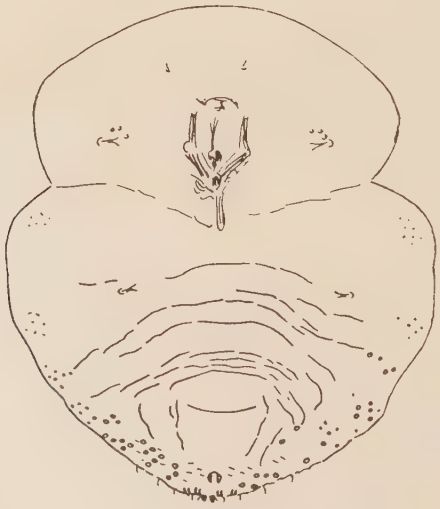

Fig. 7.

Myrtophila adnatae. Outline of body (85X).

Fig. 8.

Myrtophila adnatae. Enlargement of pygidium (340X).

Notes.—Sometimes there are indications of chitinous points in the positions of the second and third pairs of lobes but so small as to be disregarded as lobes. The distance between the median lobes varies from one-quarter to two-thirds of a lobe's width, the subapical area may not be indented and the apex blunt or but slightly rounded. The lobes may be slightly convergent. Plates in the median interlobal space may be indistinct.

The narrower median lobes, apparent absence of the second and third pairs of lobes, and the presence of ventral palisade chitinization are characters separating this species from *M. curvata* and *M. suticollis; M. curvata* has a lateral thoracic spur. *M. suticollis* has the spines near the median lobes much longer than the lobes and three pairs of definite lobes.

Type Reg. No. T. 5619 and paratypes Nos. T. 5620 to T. 5622 in the Queensland Museum.

Myrtophila suticollis n.sp.

(Figs. 9 and 10.)

Locality and host.—Queensland: Amberley on *Melaleuca irbyana* R. T. Baker, July 1954.

Habit.—Insects single and sparse on the minute host leaves. Scale of female circular, 1·2 mm. diameter; dark fawn in colour, pellicles paler.

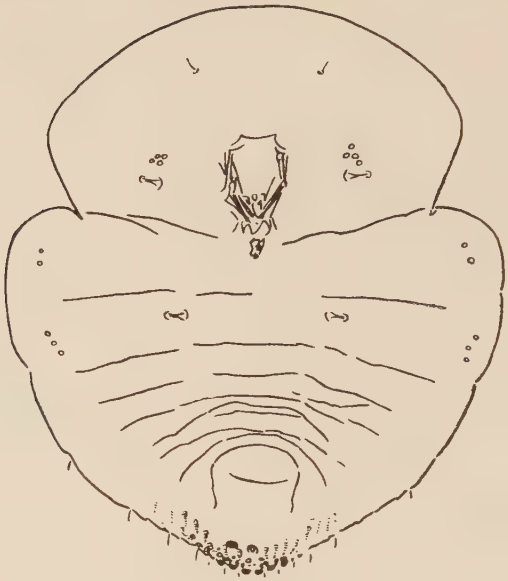

Fig. 9.
Myrtophila suticollis. Outline of body (95X).

Fig. 10.
Myrtophila suticollis. Enlargement of pygidium (340X).

Recognition characters.—Adult female with a deep thoracic constriction; anterior portion dome-shaped, lateral margins posteriorly convergent; posterior portion wider, semicircular. Length of slide specimens 0.8 mm., width 0.7 mm. Pygidium not differentiated, very broad, apex broadly rounded. Three pairs of lobes. Median lobes wider than long, entire, inner and outer margins slightly curved, almost parallel, apex broadly curved, separated by a lobe's width. Second pair of lobes slightly smaller than the median, wider than long, inner margins almost straight, outer and apical margins broadly curved, separated from the median by slightly more than the median's width. Third pair of lobes similar to but smaller than the second, separated by more than twice the latter's width. Pygidial margin with a small chitinized triangular lobe-like tooth just beyond the third lobe with other small points anteriorly. Basal scleroses absent. Paraphyses small, as a pair of short stout thickenings in each of the first and second interlobal areas. Plates in the median, first and second interlobal spaces branched, longer than the lobes, two in the median and first spaces, three in the second space and an incipient plate beyond the third lobe, the outer plate in each of the first and second spaces broader and fimbriate. Spines two to three times as long as the lobes. Dorsal ducts short, extending to the anal opening in the median area, of different sizes, a large one in the median area, two large ones in the first duct furrow, one or two large and one small in the second furrow, a large, a medium and several small ducts in the third furrow, other ducts in segmental series, small and few in number. Perivulvar pores absent. Anal opening small, three times the median lobe's length from the bases of the lobes. Vulva wide. Dorsal surface of pygidium with a broad area of reticulate chitinization, remainder of body with a finger-print chitinization. Anterior spiracles with four to six associated pores. Thoracic spur absent.

Notes.—The definite second and third pairs of lobes, the smaller number of dorsal ducts and the absence of thickening or palisade chitinization on pre-pygidial segments are characters separating this species from *M. curvata* and *M. adnatae.*

Type Reg. No. T. 5623 and paratypes Nos. T. 5624 to T. 5625 in the Queensland Museum.

Dichosoma new genus.

Genotype.—Dichosoma convexa n.sp.

Characters.—Adult female with a thoracic constriction, anterior portion dome-shaped, posterior portion semicircular. Pygidium with median lobes only, these united. Basal scleroses absent. Paraphyses small in the first interlobal area and sometimes evident in the second interlobal area. Plates prominent, mostly branched. Spines long. Duct orifices mostly marginal. Perivulvar pores absent. Dorsal pygidial area with a fine reticulate to stipple-like chitinization. Parastigmatic pores absent.

Notes.—The genus *Dichosoma* resembles *Neomorgania* and *Mimeraspis* in having united median lobes. It differs from both of these genera, however, in having long spines and a greater number of plates which are prominent, and in the absence of parastigmatic pores.

Dichosoma convexa n.sp.

(Figs. 11 and 12.)

Locality and host.—Queensland, Mt. Isa, on *Eucalyptus microtheca* F. Muell., May, 1946.

Fig. 11.

Dichosoma convexa. Outline of body (180X).

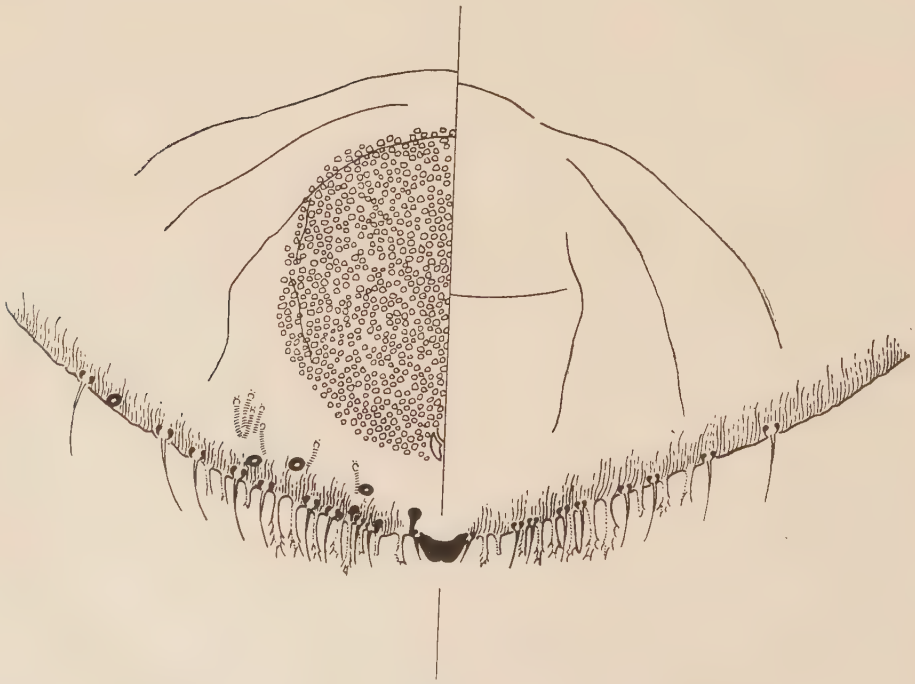

Fig. 12.

Dichosoma convexa. Enlargement of pygidium (380X).

Habit.—Insects numerous on leaves. Scales convex, circular, 0·5 mm. diameter, fawn in colour and centrally surmounted by dark greenish black pellicles.

Recognition characters.—Adult female subcircular, with a thoracic constriction; anterior portion dome-shaped, twice as wide as long; posterior portion wider, semicircular. Length of slide specimen 0.45 mm., width 0.4 mm. Pygidium wide, apex broadly rounded. Median pair of lobes only, united, combined width one-and-one-half times the length, outer margins almost straight and strongly convergent, apex truncated with a slight median concavity associated with a short subapical median line. Basal scleroses absent. Paraphyses as uneven pairs in the first and second interlobal areas, the inner paraphysis in the first interlobal area as long as the lobes, and enlarged towards the apex; inner paraphysis in the second interlobal area similar to that of the first but smaller; outer paraphysis in each interlobal area small and indistinct. Eleven plates alternating with the spines (dorsal and ventral) 2, 1, 2, 1, 2, 1, 2, mostly longer than the lobes, of a similar general erect and branched pattern except that the second or first and second near the lobes and the ninth may be simple. Spines adjacent to the lobes as long as the lobes, other spines progressively longer and becoming hair-like, then shorter on the anterior abdominal segments. Ducts slender, of moderate length, orifices marginal and submarginal. Perivulvar pores absent. Anal opening small, twice the lobe's length distant from the bases of the lobes, in an inverted U. Parastigmatic pores absent. Pygidial dorsal surface with a finely reticulate chitinization. Posterior margin of pre-pygidial abdominal segments with a fine palisade chitinization.

Notes.—The lobes may sometimes have the apices slightly rounded with the median indentation more noticeable. The paraphyses in the second interlobal area are mostly indistinct. The pairs of abdominal segmental spines are separated and alternate with the plates as mentioned above. The chitinized pygidium may be ventrally concave, which makes good slide mounting difficult. The body generally may be chitinized but not necessarily in an obvious finger-print pattern. Submarginally duct orifices may be indistinct, possibly due to heavy chitinization, but there are six or more ducts in each of the second and third duct furrows.

Type Reg. No. T. 5626 and paratypes Nos. T. 5627 to T. 5629 in the Queensland Museum.

Achorophora new genus.

Genotype.—Achorophora obliqua n.sp.

Characters.—Adult female with a deep thoracic constriction, anterior portion dome-shaped, posterior portion subtriangular. Pygidium with three pairs of lobes not uniformly shaped, pygidial margin serrate anterior to the third lobes. Basal scleroses absent. Paraphyses in the first and second interlobal areas. Plates in at least the first interlobal space. Dorsal ducts long, slender, orifices oblique. Perivulvar pores absent. Pores associated with anterior spiracles. Body at maturity heavily chitinized.

Notes.—Achorophora resembles several other genera such as *Pseudaonidia* in having a constricted thorax, a chitinized body and parastigmatic pores. The dorsal pygidial chitinization has branched sutures resembling those of *Neoleonardia.* The oblique duct orifices are different from all other Australian genera in the *Pseudaonidia* group.

Achorophora obliqua n.sp.

(Figs. 13 and 14.)

Locality and host.—Queensland: Drillham, on *Casuarina luehmannii* R. T. Baker, Apr. 1953 (J. Mann).

Habit.—Insects on branchlets, single or several adjacent to each other with the anterior end close to or partly under the whorl of leaflets. Scale oval with pellicles dark orange.

Recognition characters.—Adult female with a thoracic constriction; anterior portion dome-shaped, length two-thirds the width, lateral margins posteriorly slightly convergent; posterior portion wider, subtriangular, longer than wide, apex slightly acute. Length of slide mounted specimen 0.7 mm., width 0.5 mm. Pygidium comparatively large, not differentiated, apex rounded; three pairs of definite lobes and chitinized serrations anteriorly. Median lobes longer than wide, inner margins slightly convergent, outer margins curved; the rounded top of apex narrowly separating subapical indentations. Second pair of lobes slightly longer than wide, inner margin almost straight, outer margin broadly curved, apex rounded with indefinite indentations, separated from the median by the width of the median. Third pair of lobes as wide or wider than long, inner margin almost straight, outer margin with apex forming a broadly curved latero-apical margin with three indentations, separated from the second lobes by slightly less than the width of the median. Basal scleroses absent. Paraphyses slender, as an uneven pair in the first and second interlobal areas, the inner in each area as long as the median lobes. Plates as long as the lobes, bifid, two in the first, one in the second interlobal space. Spines adjacent to the lobes as long as the lobes, others shorter. Dorsal ducts long in the median area, longer than the distance from the lobes to the anal opening, two in the median area, five or six in the first duct furrow, six in the second, others becoming shorter and smaller anteriorly; orifices oblique to the median line, mostly marginal and submarginal. Perivulvar pores absent. Anal opening small in an inverted U, with an anal groove, five times the length of the median lobes distant from the bases of the lobes. Vulva large, twice as far from the lobes as the anal opening. Dorsal pygidial chitinization not a pronounced reticulation, elsewhere body with a finger-print chitinization. Anterior spiracles with two associated pores.

Fig. 13.

Achorophora obliqua. Outline of body (100X).

Fig. 14.

Achorophora obliqua. Enlargement of pygidium (290X).

Notes.—In some specimens the anterior portion of the body is only slightly more than half as long as wide, and the lateral margins may be almost parallel. The indentations on the second and third lobes may be indistinct. Some individuals show two simple plates in the median interlobal area. Others may have the median lobes almost contiguous. Ducts are more evident by duct heads than by orifices due to chitinization. Ventral duct orifices are oblique. The oblique disposition of duct orifices is also distinct in second-stage specimens.

Type Reg. No. T. 5630 and paratypes Nos. T. 5631 to T. 5633 in the Queensland Museum.

Achorophora divergens n.sp.

(Figs. 15 and 16.)

Locality and host.—Queensland: Marmor on *Casuarina glauca* Sieb., Oct. 1955.

Habit.—Insects singly or in small groups with anterior of scale downwards into whorl of leaflets. Scale oval, length 1·8 mm., width 1·3 mm., sometimes partly curved around the branchlet, dark reddish brown or brownish black; pellicles dark orange, placed near the anterior end of scale.

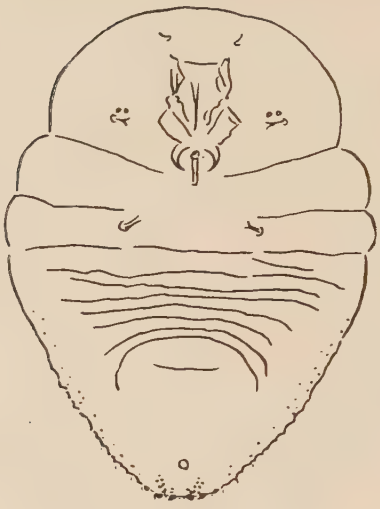

Fig. 15.

Achorophora divergens. Outline of body (50X).

Fig. 16.

Achorophora divergens. Enlargement of pygidium (255X).

Recognition characters.—Adult female broadly oval, with a thoracic constriction; anterior portion dome-shaped, lateral margins slightly convergent posteriorly; posterior portion wider, as wide or wider than long. Length of slide mounted specimen 1.5 mm., width 1.1 mm. Pygidium comparatively large, not differentiated, apex broadly rounded; three pairs of lobes and chitinized serrations anteriorly. Median lobes of unusual shape, widely divergent. Second pair of lobes longer than wide, convergent, separated from the apparent outer margin of the median lobes by the second lobe's width. Third pair of lobes as wide as long, apex broadly rounded, separated from the second by the third lobe's width. Basal scleroses absent. Paraphyses as a pair in the first and second interlobal areas, longer than the second lobes. Spines short. Plates apparently in the first interlobal space but shape obscure. Dorsal ducts slender, long, in the first furrow two-and-a-half times the distance between the lobes and anal opening, longer in the second furrow; orifices oblique, one or two in the median interlobal area, about five in the first furrow, eight to 15 in the second furrow. Perivulvar pores absent. Anal opening small, in an inverted U, four to six times the length of the second lobes from the bases of the median lobes. Anterior spiracles with two associated pores. Body heavily chitinized.

Notes.—Fig. 15 shows the unusual nature of the median lobes. The two short broad truncated median structures may be plates but appear to be chitinized. It is also difficult to determine whether the paraphyses are not chitinized extensions of the lateral margins of the lobes. The third pair of lobes resembles adjacent chitinized marginal serrations, but are regarded as lobes because of the definite appearance as such in the second-stage females and in conformity with the other species, *A. obliqua.* The numbers of duct orifices vary, determined mainly on the number of duct heads, which mostly are goblet-shaped, with a long flagellum. Pores associated with the anterior spiracles may vary up to four. Ventral ducts resemble the dorsal ducts but are smaller and become progressively smaller anteriorly; orifices marginal or submarginal.

The widely divergent median lobes distinguish this species from *A. obliqua.*

Type Reg. No. T. 5634 and paratypes Nos. T. 5635 to T. 5637 in the Queensland Museum.

Diaphoraspis new genus.

Genotype.—Diaphoraspis orbata n.sp.

Characters.—Adult female with a deep thoracic constriction, anterior portion dome-shaped, posterior portion semicircular to broadly shield-shaped. Pygidium with three pairs of lobes, not uniformly shaped. Median lobes almost contiguous or divergently separated. Basal scleroses absent. Paraphyses in the first and second interlobal areas. Plates absent. Dorsal ducts slender, often with a flagellum apparent beyond the duct head, orifices small. Perivulvar pores absent. Dorsal pygidial chitinization like a stippling and with widely separated and branched sutures on mature specimens. Anterior spiracles with associated pores.

Notes.—Diaphoraspis resembles *Achorophora* in having a constricted thorax, parastigmatic pores and sutures in the dorsal pygidial chitinization, but the lobes are quite different and the duct orifices are not oblique.

Diaphoraspis orbata n.sp.

(Figs. 17 and 18.)

Localities and hosts.—Queensland: type and paratype from Moggill on *Casuarina cunninghamiana* Miq., July 1953; other paratypes from Drillham on *Casuarina luehmannii* R. T. Baker, Apr. 1953 (J. Mann).

Habit.—Insects single on twigs mostly in axils of the branchlets. Scales subcircular, 1·2 mm. diameter, or slightly oval; dark to blackish brown, pellicles brown.

Recognition characters.—Adult female with a deep constriction between the mesothorax and metathorax and a smaller constriction between the metathorax and the abdomen; anterior portion dome-shaped, lateral margins posteriorly divergent; posterior portion wider, broadly shield-shaped. Length of slide specimen 0.8 mm., width 0.7 mm. Pygidium large, apex rounded. Three pairs of lobes. Median lobes longer than wide, narrowed basally, inner and outer margins concave, apex broadly rounded, with one or two indefinite small apical indentations, separated by one-third of a lobe's width. Second pair of lobes convergent, longer than wide, narrowed basally, apex rounded with indefinite indentations, distally contiguous with the median lobes. Third pair of lobes as wide as long, with a small dome-shaped apex between apical indentations, separated from the second lobes by half of the width of the latter. Basal scleroses absent. Paraphyses as elongate pairs in the first and second interlobal areas, the inner paraphysis in each interlobal area slightly longer than the median lobes. Plates absent. Spines adjacent to the median lobes shorter than the lobes, other spines subequal in length to the median lobes. Ducts slender with heads longer than wide, and somewhat cylindrical, in the median area long and very slender extending to three-quarters of the distance between the lobes and vulva with a flagellum four or more times the length of the heads, becoming progressively shorter in anterior duct furrows; orifices in segmental series, those within the paraphyses and on the chitinized marginal rim indiscernible, otherwise six to eight discernible in the second furrow, 10 in the third, 15 in the fourth and less in the fifth. Perivulvar pores absent. Anal opening

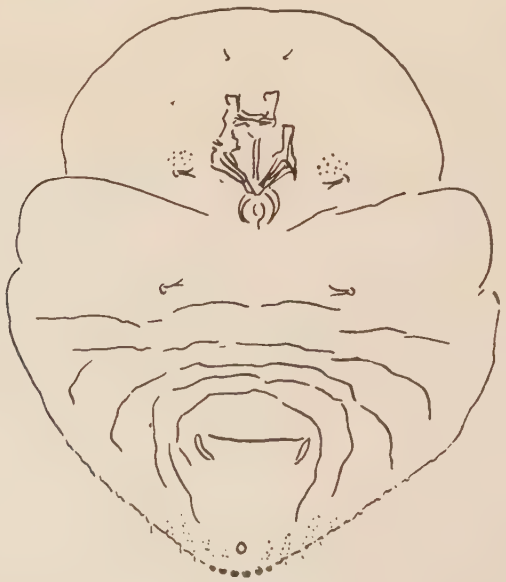

Fig. 17.

Diaphoraspis orbata. Outline of body (95X).

Fig. 18.

Diaphoraspis orbata. Enlargement of pygidium (210X).

small, in an inverted V, twice the length of the median lobes distant from the bases of the lobes, with an anal groove extending between the bases of the median lobes. Vulva wide. Margin anterior to the third lobes finely serrate. Anterior spiracles with eight to 11 associated pores.

Notes.—In some specimens the lateral margins of the anterior portion of the body may be almost parallel. The heavy chitinization may make the median lobes appear to be basally yoked, but an anal groove is still evident. This area is heavily chitinized, as is most of the pygidial margin. The pores associated with the anterior spiracles may be as few as four. A narrow median groove extends ventrally from near the vulva to between the median lobes. Duct orifices in the second and third furrows extend anteriorly near segmental folds for one-third to one-half of the distance from the margin to where these folds arch in the pre-pygidial region. Ventral duct orifices submarginally on the pygidium are numerous. Pygidial chitinization is like a stippling on both the dorsal and the ventral surfaces.

Type Reg. No. T. 5638 and paratypes Nos. T. 5639 to T. 5641 in the Queensland Museum.

Diaphoraspis incisa n.sp.

(Figs. 19 and 20.)

Locality and host.—Queensland: Moggill, on *Casuarina cunninghamiana* Miq., July 1953.

Fig. 19.

Diaphoraspis incisa. Outline of body (70X).

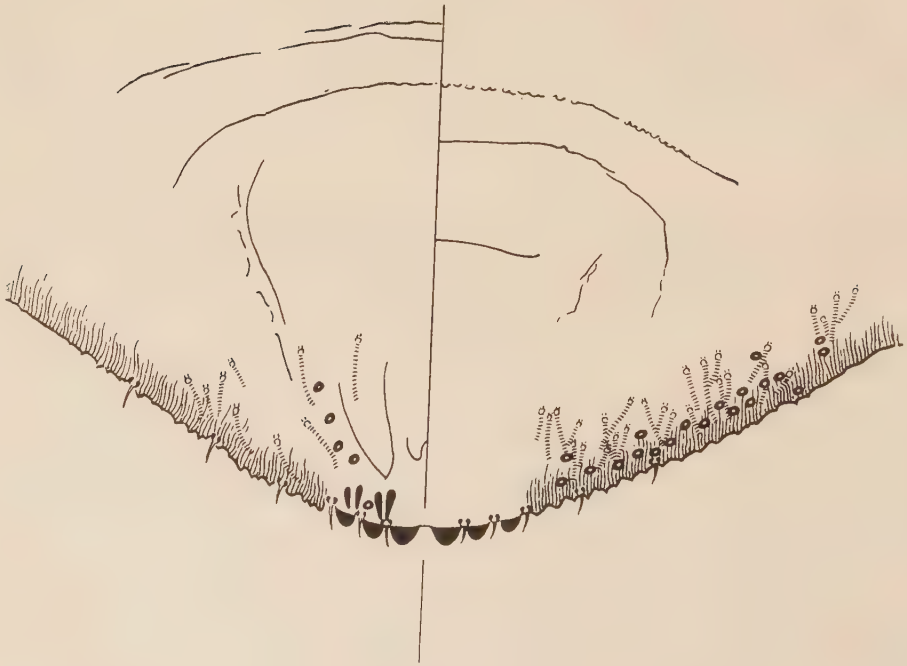

Fig. 20.

Diaphoraspis incisa. Enlargement of pygidium (255X).

Habit.—Insects single on twigs mostly in axils of branchlets or leaflet whorls. Scale subcircular, 1·5 mm. diameter, convex, hard, black with a brown loose suffusion; pellicles near the centre, reddish black.

Recognition characters.—Adult female subcircular, with a deep thoracic constriction, anterior portion dome-shaped; posterior portion wider, semicircular. Length of slide mounted specimen 1.0 mm., width 0.9 mm. Pygidium comparatively large, not differentiated, apex broadly rounded; three pairs of lobes and margin serrate anteriorly. Median lobes yoked basally, as wide as long, inner margins widely divergent; inner and outer margins similar, convergent to the broadly rounded apex; separated basally by half a lobe's width. Second and third pairs of lobes similar but smaller, second separated from the median by one-quarter of the latter's width and third from the second by half its own width. Basal scleroses absent. Paraphyses as uneven pairs in the first and second interlobal areas, the inner paraphysis in each area as long or slightly longer than the median lobes. Plates absent. Spines as long as the lobes. Dorsal ducts sparse. Perivulvar pores absent. Anal opening small in an inverted V, twice the length of the median lobes distant from the bases of the lobes. Anterior spiracles with six or seven associated pores.

Notes.—Dorsal pygidial chitinization essentially plain but ventrally is like a fine stippling, elsewhere there is the typical fingerprint chitinization, which may be dense. The constriction between the metathorax and first abdominal segment is not such a conspicuous feature as in *A. orbata* and *A. compacta.* In some instances there are indications of small subapical indentations on the lobes. Median lobes appear to be chitinously yoked basally. The dorsal ducts are inconspicuous; the only obvious orifices are four or five in the second duct furrow. There are, however, numerous ducts with orifices marginal and submarginal on the ventral surface.

This species may be separated from the other two in the genus by the stouter, shorter and widely separated median lobes, the second lobes are not convergent to each other and the median and second lobes are not contiguous. The dorsal ducts are sparse.

Type Reg. No. T. 5642 and paratypes Nos. T. 5643 to T. 5645 in the Queensland Museum.

Diaphoraspis compacta n.sp.

(Figs. 21 and 22.)

Locality and host.—Queensland: Forest Hill, on *Casuarina cunninghamiana* Miq., Jan. 1953.

Habit.—Insects scattered on twigs or in axils of leaflet whorls. Scales broadly oval to subcircular, 1·5 mm. long, fawn to grey; pellicles towards the anterior end, orange to dark yellow.

Recognition characters.—Adult female broadly oval, with a deep thoracic constriction between the mesothorax and metathorax and a secondary constriction between the metathorax and the first abdominal segment. Anterior body portion dome-shaped, lateral margins concave and posteriorly convergent, a stout spur on the lateral angle. Metathorax wider than the anterior portion, lateral margins convex but divergent. Posterior portion widest, almost semicircular. Length of slide mounted specimen 1.0 mm., width 0.75 mm. Pygidium broad, not differentiated; three pairs of lobes and lobe-like chitinous points anteriorly. Median

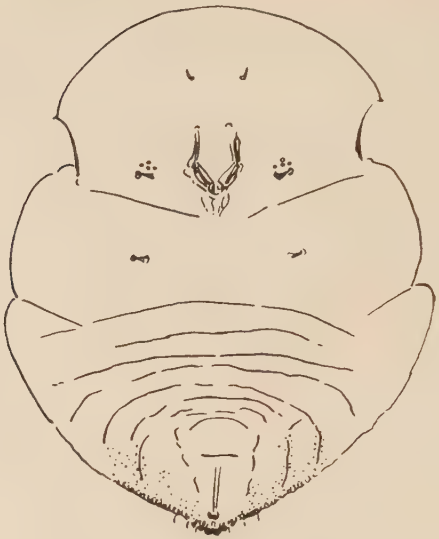

Fig. 21.

Diaphoraspis compacta. Outline of body (70X).

Fig. 22.

Diaphoraspis compacta. Enlargement of pygidium (255X).

lobes as wide as long, widest across the middle, inner margins almost straight, slightly convergent, outer margins slightly curved and divergent, apex very broadly rounded with indications of small indentations, separated basally although appearing yoked, but almost contiguous subapically. Second pair of lobes convergent, as wide as long, outer margins broadly curved, almost contiguous with the median lobes. Third pair of lobes wider than long, apex broadly rounded, separated from the second by the third lobe's width. Basal scleroses absent. Paraphyses as pairs in the first and second interlobal areas, the inner in each area almost as long or as the median lobes. Plates absent. Spines adjacent to the median and second lobes shorter than the lobes, other spines subequal in length to the median lobes. Dorsal ducts slender, in the median region four times the length of the median lobes, orifices small, two in the median interlobal area, about six in the first duct furrow, about 10 in the second furrow, 15 in each of the third and fourth furrows, and some in the fifth; in the third, fourth and fifth furrows duct orifices extending to the region where the segmental fold arches anteriorly on the pygidium. Perivulvar pores absent. Anal opening small, one-and-a-half times the length of the median lobes distant from the bases of the lobes, with an anal groove between the bases of the lobes. Anterior spiracles with four or five associated pores.

Notes.—The pygidial chitinization both dorsally and ventrally may be like stippling. A narrow ventral groove extends from near the vulva to between the median lobes. Ventral ducts with marginal and submarginal orifices are numerous. Median lobes may be longer than wide and may appear to be chitinously yoked basally. The pores associated with the anterior spiracles may vary up to seven. Chitinization obscures the dorsal duct orifices and the number of ducts may be more evident by duct heads. Some specimens, perhaps not so mature as the type, have the anterior portion less upraised and in some instances comparable to that of *D. orbata*. Body chitinization is dense.

The presence of lateral thoracic spurs, the greater number of ducts and their extension anteriorly are features separating this species from *D. orbata* and *D. incisa*.

Type Reg. No. T. 5646 and paratypes Nos. T. 5647 to T. 5649 in the Queensland Musueum.

Aspidonymus new genus.

Genotype.—Aspidonymus woodwardi n.sp.

Characters.—Adult female with a deep thoracic constriction, anterior portion dome-shaped, posterior portion deltoid. Pygidium with three pairs of lobes of similar shape. Basal scleroses absent. Paraphyses as small yoked pairs in the median, first and second interlobal areas. Plates in the median, first and second interlobal spaces. Dorsal ducts long and slender. Perivulvar pores absent. Anterior spiracles with associated pores. Dorsal pygidial chitinization finely reticulated. Body of mature females heavily chitinized.

Notes.—The genus *Aspidonymus* resembles *Pseudaonidia* in having the constricted thorax and similarly shaped pygidial lobes. The lobes and the plates, however, are much more slender, and the body is smaller and more slender in shape. The ducts also are more slender and the pygidial chitinization much finer.

Aspidonymus woodwardi n.sp.

(Figs. 23 and 24.)

Locality and host.—Queensland: Beenleigh on *Dissilaria baloghioides* F. Muell. ex Baill., May 1956.

Habit.—Insects sparse on the undersurface of leaves. Scale circular, 1·5 mm. diameter, pale to light brown; pellicles light yellow to orange.

Recognition characters.—Adult female broadly pyriform with a deep thoracic constriction; anterior portion dome-shaped, lateral margins posteriorly convergent; posterior portion wider, broadly deltoid. Length of slide specimen 0.8 mm., width 0.6 mm. Pygidium large, triangular, apex broadly rounded. Three pairs of lobes. Median lobes two to two-and-a-half times longer than wide, widest at the middle, with an indentation on both the inner and outer margins just beyond the middle, apex rounded, separated by one-and-a-half times a lobe's width. Second pair of lobes two to two-and-a-half times as long as wide, similar in shape to the median lobes but without the indentation on the inner margin, separated from the median lobes by one-and-a-half times the latter's width. Third pair of lobes similar in shape and size to the second, separated by two-and-a-half times the width of the second. A large lobe-like chitinous tooth, wider than long, in the position of the fourth lobe, with two or three indentations on the outer margin. Basal scleroses absent. Paraphyses as small yoked pairs in the median, first and second interlobal areas. Median interlobal space indented beyond the body margin. Plates in the median, first, second and third interlobal spaces. Two plates in the median space, two in the first space and three in the second space, of similar shape and size, as long as the lobes, bifid with short arms; the first two plates in the third space broader and fimbriate on the outer margin, the third plate in this space bifid with long arms. Spines adjacent to lobes shorter than the lobes. Dorsal ducts long, slender, extending to the anal opening in the median area; orifices small, one in the median area, two in the first interlobal area, others not distinctly in furrows, but five or more in the second furrow, 10 or more in the third, others on anterior abdominal segments with the ducts becoming shorter. Perivulvar pores absent. Anal opening oval,

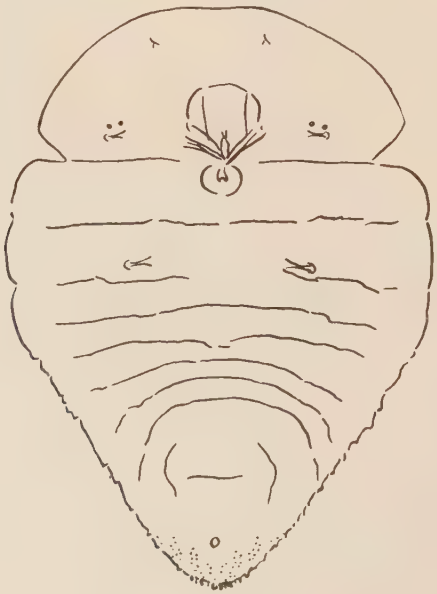

Fig. 23.

Aspidonymus woodwardi. Outline of body (95X).

Fig. 24.

Aspidonymus woodwardi. Enlargement of pygidium (210X).

four times its long axis from the bases of the median lobes. Vulva wide. Dorsal surface of pygidium heavily chitinized. Two pores associated with anterior spiracles.

Notes.—A duct in each of the first and second furrows with a marginal orifice is broader and has a larger head than other ducts. In some specimens the median first and second pairs of lobes are of comparable shape and size. The structure in the position of the fourth lobe has the appearance of a lobe but is quite different in shape. Old specimens have a finger-print chitinization on the body. Named after Dr. T. E. Woodward, Department of Entomology, University of Queensland, who made the collection of the material possible.

Type Reg. No. T. 5650 and paratypes Nos. T. 5651 to T. 5653 in the Queensland Museum.

Acontonidia new genus.

Genotype.—Acontonidia triangularis n.sp.

Characters.—Adult female with a deep thoracic constriction, anterior portion dome-shaped, posterior portion subtriangular to broadly shield-shaped. Pygidium with three pairs of lobes of a comparable pattern in shape but differing greatly in size. Basal scleroses absent. Paraphyses distinct and as pairs in the median first and second interlobal areas. Plates slender and present in the median first and second interlobal spaces. Dorsal ducts long and slender. Perivulvar pores absent. Abdominal margin crenulate to serrate. Dorsal pygidial area heavily chitinized. Anterior spiracles with associated pores.

Notes.—The genus *Acontonidia* has some resemblance to *Aspidonymus* but the nature of the lobes, plates, paraphyses and abdominal margin is quite different.

Acontonidia triangularis n.sp.

(Figs. 25 and 26.)

Type locality and host.—Queensland: Beenleigh, on *Dissilaria baloghioides* F. Muell. ex Baill., May, 1956.

Habit.—Insects single and sparse, embedded under cork tissue on twigs.

Recognition characters.—Adult female broadly pyriform, with a deep thoracic constriction; anterior portion dome-shaped, lateral margins posteriorly sharply convergent; posterior portion of the same width, almost equilateral, lateral margins slightly curved. Length of slide specimens 0.8 mm., width 0.7 mm. Pygidium comparatively large, apex broadly rounded. Three pairs of lobes. Median lobes as wide as long, inner and outer margins similar, convergently curved to large subapical indentations, apex dome-shaped, separated by one-third of a lobe's width. Second pair of lobes much smaller, outer margin longer than the inner, with a subapical indentation; the inner margin slightly curved, with a small subapical indentation, separated from the median by one-quarter of the median lobe's width. Third pair of lobes much smaller than the second, margins curved to a triangular point, separated from the second by twice the latter's width. Basal scleroses absent. Paraphyses in the median first and second interlobal areas; in the median area as long as or slightly longer than the median lobes and divergent; in the first area as an uneven pair, the outer longer; similar but smaller in the second area. A pair of faint slender simple plates in the median, first and second interlobal spaces, as long as the lobes. Spines adjacent to the median lobes shorter than the lobes, other species longer. Dorsal ducts few, long and slender, orifices small, two or three in the first furrow, four or five in the second. Perivulvar pores absent. Anal opening small, four times the length of the median lobes from the bases of the lobes, apparently with an internal anal tube, and

Fig. 25.

Acontonidia triangularis. Outline of body (80X).

Fig. 26.
Acontonidia triangularis. Enlargement of pygidium (255X).

with an anal groove between the median paraphyses. Vulva very wide. Abdominal margin beyond the third pair of lobes crenulate, almost serrate. Median pygidial area with chitinized reticulation, body elsewhere with a finger-print chitinization. Anterior spiracles with six or seven associated pores.

Notes.—The scale is brittle and breaks into small pieces as the insect is extracted and the shape and colour are difficult to determine. Paraphyses in the median interlobal area may be enlarged anteriorly. Slight thickenings on the outer and inner basal areas of the median lobes may give the impression of a third paraphyses in the first interlobal area and a small second pair in the median area. Sometimes the third lobes are only small triangular points.

Type Reg. No. T. 5654 and paratypes Nos. T. 5655 to T. 5657 in the Queensland Museum.

Duplaspidiotus magnus n.sp.

(Figs. 27 and 28.)

Type locality and host.—Queensland: Tugun on *Melichrus urceolatus* R.Br., Jan. 1950.

Habit.—Insects mostly single near ground level on trunk of host, almost embedded in cork tissue. Scale circular to slightly oval, 2·3 mm. diameter, dark brown to brownish grey; first pellicle light brown.

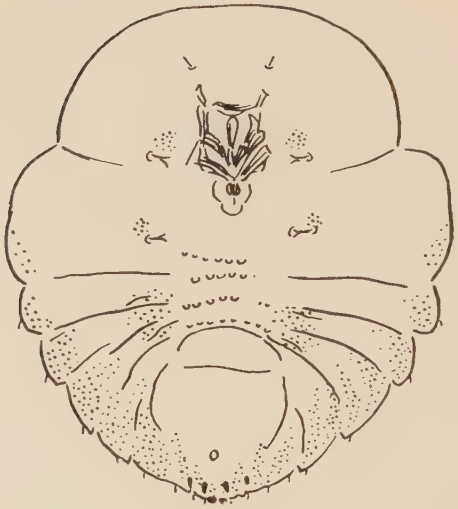

Fig. 27.

Duplaspidiotus magnus. Outline of body (55X).

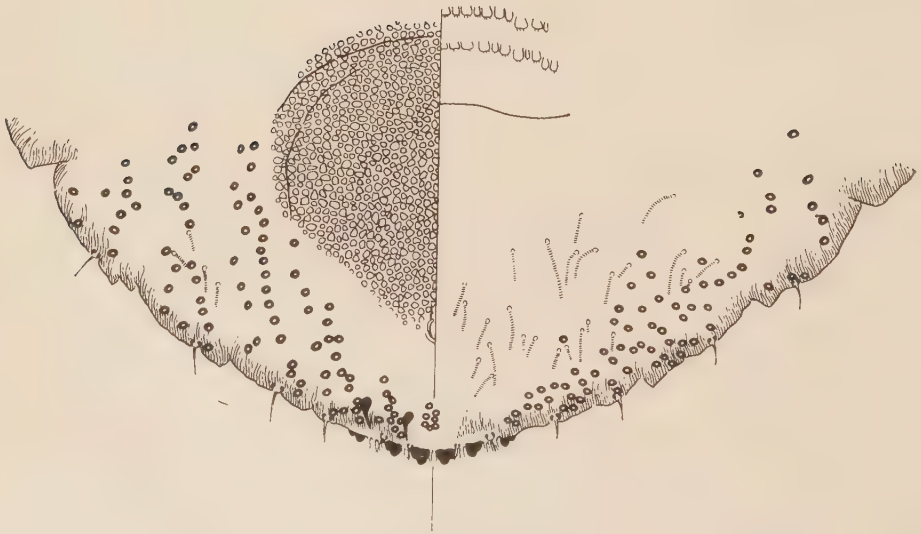

Fig. 28.

Duplaspidiotus magnus. Enlargement of pygidium (160X).

Recognition characters.—Body of adult female broadly oval with a thoracic constriction, and abdominal segmental demarcations. Length of slide specimen 1.4 mm., width 1.05 mm.; anterior portion dome-shaped, posterior portion wider, somewhat semicircular. Pygidium broadly rounded, not demarcated. Three pairs of lobes. Median lobes as wide as long, separated by a third of a lobe's width, inner margin straight or slightly curved, with a small basal constriction and a subapical indentation; outer margin longer than the inner, broadly curved, with a large subapical indentation; apex rounded. Second pair of lobes similar to the median but half the size and without the inner subapical indentation, separated from the median lobes by one-third to one-half of the latter's width. Third pair of lobes similar to the second but smaller and more triangular, separated from the first by one-and-a-third times the latter's width. Basal scleroses absent. Paraphyses in the first and second interlobal areas, single, as long as or slightly longer than the median lobes, apically clubbed. Plates shorter than the lobes, simple, two in the median interlobal space, two in the first interlobal space, and three in the second. Spines adjacent to the median lobes as long as the lobes, other spines longer. Ducts numerous, slender, heads small, length in the median region half the distance from the lobes to the anal opening; orifices in segmental series, in double rows on most abdominal segments, seven in the median interlobal area, 12 to 15 in the first duct furrow, 20 to 24 in the second, more numerous in other furrows but only eight submarginally on the metathorax; submedian series of ducts dorsally on anterior abdominal segments. Perivulvar pores absent. Anal opening small, oval, five times the length of the long axis from the bases of the median lobes. Thirty to 35 anterior parastigmatic pores; nine to 12 posterior parastigmatic pores. Median pygidial area with heavy tessellated chitinization. Abdominal segments 1 to 6 with ventral median bands of palisade chitinization.

Notes.—In some specimens the plates are little more than short conical derm projections, and in old specimens the lobes have only slight indentations. The species may be distinguished from other species in the genus in having ventral bands of palisade chitinization on the abdominal segments.

Type Reg. No. T. 5658 and paratypes Nos. T. 5659 to T. 5661 in the Queensland Museum.

Pseudotargionia inconspicua n.sp.

(Figs. 29 and 30.)

Locality and host.—Queensland; Rocklea on *Melaleuca leucadendra* (L.) L., Sept. 1914 (H. Tryon).

Habit.—Insects scattered on twigs; scale beneath a thin layer of cork tissue, round, 1·6 mm. diameter, undersurface whitish; pellicles brown.

Recognition characters.—Adult female broadly oval, with a deep thoracic constriction; anterior portion dome-shaped with lateral margins strongly convergent posteriorly; posterior portion slightly wider, semicircular. Length of slide mounted specimen 1.0 mm., width 0.85 mm. Pygidium wide, apex broadly rounded. One pair of lobes yoked basally, slightly wider than long, inner margins divergent with a subapical indentation, outer margins curved with a large subapical indentation, apex broadly rounded; basally separated by one-third of a lobe's width. Basal scleroses absent. Paraphyses in the first and second interlobal areas, on the inner side only, anteriorly enlarged, longer than the lobes, shorter in the second than in the first interlobal area. Spines adjacent to the lobes shorter than the lobes, other spines longer. A pair of simple truncated plates in the first and second interlobal spaces, shorter than the lobes. Dorsal ducts short and slender; orifices small with chitinized rims, a few in the first furrow, 14 in the second and about 25 in the third, with about 15 as an intervening row. Perivulvar pores absent. Anal opening small, in an inverted V, one-and-a-half

Fig. 29.

Pseudotargionia inconspicua. Outline of body (80X).

Fig. 30.

Pseudotargionia inconspicua. Enlargement of pygidium (210X).

to two times the lobe's length from the bases of the lobes. Anal groove present. Vulva wide, posteriorly curved. Pygidium dorsally with a large area of reticulate chitinization, elsewhere heavily chitinized. Pygidial margin crenulate, tending to be serrate. Anterior spiracles with four or five associated pores, posterior spiracles with two pores.

Notes.—In older specimens the pygidium has a heavily chitinized marginal rim. Heavy chitinization obscures the duct orifices and paraphyses, and on the dorsal surface the reticulate thickening may be very broad. Ducts in the second furrow may number as many as 20 with a similar number in an intervening row between the second and third furrows. Many ventral ducts are present with orifices on or near the chitinous marginal rim. Pores associated with the anterior spiracles may vary from four to six and those with the posterior spiracles from two to three. Sometimes the inner plate in the first or second interlobal space may be pointed and the lobes broader than long with the inner margins more strongly divergent.

This species resembles *P. cordata* in shape and appearance but differs in possessing plates, in the absence of dorsal ducts beyond the third furrow, and about half the number of pores associated with the anterior spiracles.

Type Reg. No. T. 5662 and paratypes Nos. T. 5663 to T. 5665 in the Queensland Museum.

ACKNOWLEDGEMENTS.

Thanks are due to Dr. A. J. Nicholson, Chief, C.S.I.R.O Division of Entomology, Canberra, for the loan of material of *Mimeraspis rotundus*. Mr. W. Manley, Departmental Illustrator, made the drawings for all the figures.

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NEW SPECIES AND RECORDS OF DACINAE *(TRYPETIDAE, DIPTERA)* FROM QUEENSLAND AND NEW GUINEA.

By A. W. S. MAY, M.Agr.Sc., Senior Entomologist, Science Branch, Division of Plant Industry.

SUMMARY.

Seven new species are described, viz., Afrodacus flavinotus, A. furvus, Melanodacus rubidus, Psilodacus exiguus, Strumeta hispidula, S. mendosa, **and** S. robiginosa.

The males of Afrodacus tigrinus **May and** Strumeta notatagena **May, previously known only from the female, are described.**

Neodacus lanceolatus **Perk. is recorded from North Queensland.**

AFRODACUS Bez.

Afrodacus flavinotus n.sp.

A small, compact, yellow and reddish brown species with contrasting black markings on thorax and abdomen, no facial spots, short thick antennae, post-sutural median vitta, pale fulvous costal cells and a broad mesopleural stripe.

Fig. 1.

Afrodacus flavinotus. (× 12·5).

[*Drawn by William Manley.*

Female—Length, excluding ovipositor, 4.5 mm.; wing 4.1 mm.

Head.—Vertical length 1.1 mm. *Frons* flattened, fulvous, paler along eye margins; length 1.3 times width; bristles: 1 *s.or.*, 2 *i.or.* *Lunule* colourless. *Antennae* fulvous, short, third segment broad and flattened; length of segments: 0.1 mm., 0.18 mm., 0.4 mm. *Face* concave, glassy, hoary along eye margins, length 0.4 mm.; antennal grooves not pronounced, no facial spot. *Genae* pale, no subocular spot; bristle brownish. *Occiput* pale fulvous; occipital row wanting. *Vertex* paler than surroundings; bristles pale brown.

Thorax.—Mesonotum as broad as long, fulvo-ferruginous blotched with fuscous, particularly behind suture; pronounced, yet narrow, black band at posterior margin. *Pleurae* pale fulvous. With the following yellow areas:—Humeri; notopleural calli; broad, parallel-sided, lateral post-sutural stripes reaching and including upper *p.sa.* bristles; a broad medial post-sutural vitta, pointed anteriorly and attaining *prsc.* bristles laterally; a broadly triangular mesopleural stripe with apex extending onto upper sternopleuron and base extending from wing base to humeral callus; 5/6 of upper and 2/3 of lower hypopleural calli (remainder black). *Scutellum* yellow with narrow black basal band. *Postnotum* black. *Bristles*: *scp.* 4, *npl.* 2, *mpl.* 1, *p.sa.* 2, *prsc.* 2, *sc.* 2, *pt.* 1, all brown; *a.sa.* wanting. *Legs* fulvous; three long bristles beneath fore femora. *Wings*: fuscous costal band occupying cell R_1, outer edge of cell R_3 and broadening after crossing vein r_{4+5} to end at m_{1+2}. This band more intensely fuscous beyond extremity of r_{2+3}. Costal cells fulvous; microtrichia over whole of second and outer margins of first; proportion of second cell to stigma 0·7:1. Above and basal to median cell fulvous. *r-m* cross vein dividing m_{1+2} in the proportion of 1.4:1. Anal streak fulvous, very short (almost wanting); length of its extension in proportion to length of $cu_1 + 1a$, 0·9:1.

Abdomen.—Yellowish except for following black markings on tergites:—A band along fore margin of third, broken medially and widening laterally to reach hind margin; a spot on each lateral hind margin of fourth; a triangular medial spot on posterior half of fifth. *Ovipositor* pale fulvous, shiny; basal segment in length equal to fifth tergite; paired barbs situated 0.5 mm. from apex of piercer.

Holotype ♀, ex lure traps in citrus, Atherton, Queensland, May, 1957. (J. Rosser.)

Type in Queensland Museum, Reg. No. T. 5603.

The general colour of the thorax and the extent of black markings on the abdomen readily separate this species from the other two closely related Queensland species, *A. furvus* n.sp. and *A. tigrinus* May. Two other species, *A. javanensis* Perk. (from Java) and *A. trilineatus* Hardy (from India), possessing three post-sutural vittae, can be separated by the colour of the mesonotum, facial markings and characteristics of the wing.

Afrodacus furvus n.sp.

A small species with a black thorax, three post-sutural vittae, brown notopleural calli, unmarked face, short thick antennae, spines beneath fore femora, infuscation on *r-m* cross vein and a bulla on the male wing.

Male—Length 4 mm.; wing 3.8 mm.

Female—Length, excluding ovipositor, 4.5 mm.; wing 4.4 mm.

Head.—Vertical length 1.1 mm.; entirely fulvous. *Frons* flattened, 1.4 times width; bristles: 1 *s.or.*, 2 *i.or.* *Antennae* very short, fulvous, third segment broad, flattened; length of segments: 0.1 mm., 0.15 mm., 0.32 mm.; arista very long, black. *Face* sunken, length 0.3 mm.; no facial spots. *Genae* with no subocular spot; bristle black. *Occipital row* wanting. (Note: Occiput of female brownish with paired triangular black areas medially.)

Thorax.—As for *tigrinus* except notopleural calli brownish, darker on the anterior portion. *Legs* fulvous except hind tibiae brownish; three well developed bristles beneath apical half of fore femora. *Wings*: *Male*:—Without a continuous costal band. Costal cells dark fulvous; stigma fuscous; dark fulvous basal to and immediately above median cell, also in basal portions of cells R_1 and R_3 below stigma; dark fulvous in cell R.

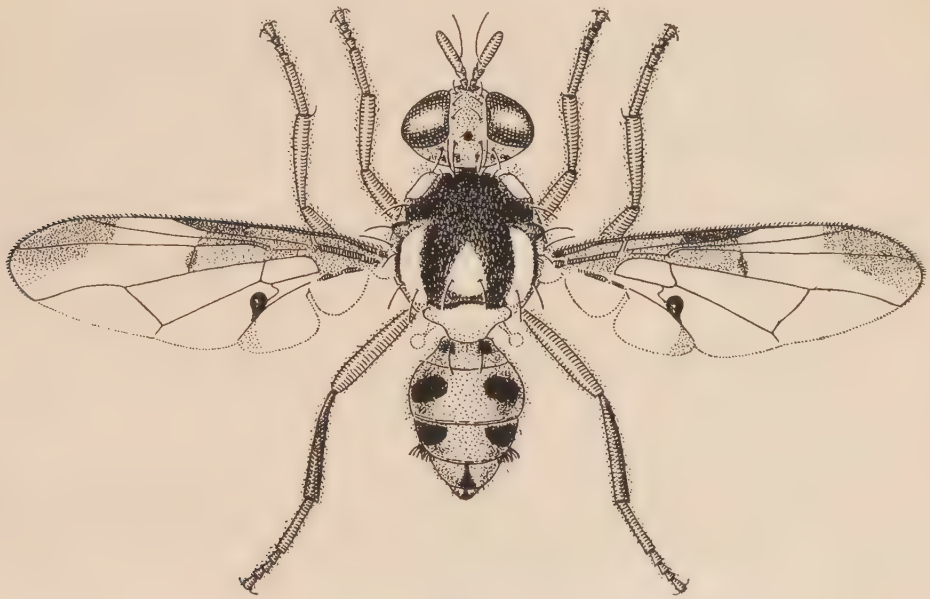

Fig. 2.

Afrodacus furvus. (× 14).

[*Drawn by William Manley.*

A fuscous semicircular apical spot occupying outer edge of cell R_1, outer third of cell R_3 and fore outer portion of cell R_5. Microtrichia covering all of wing, including costal cells, with exception of posterior half of median cell. Second costal cell to stigma in the proportion of 0.8:1. *r-m* infuscated, the proportion of m_{1+2} before and after it 1.2: 1. Anal streak lacking; length of anal cell extension to $cu_1 + 1a$ in proportion of 2.5: 1. A well developed bulla on cubital vein. Supernumerary lobe well developed. Dense aggregation of microtrichia at end of cu_1+1a. *Female*:—Costal cells fulvous, second paler than first. Costal band fuscous, occupying all of cell R_1, outer margin of cell R_3 and fading to end midway between veins r_{4+5} and m_{1+2}. Proportion of anal cell extension to $cu_1 + 1a$, 0.7: 1. Other measurements as for male wing.

Abdomen.—As for *tigrinus* except black areas more extensive, and in addition on male, paired black spots on fore margin of first tergite around each lateral hump; apices of triangular shaped medial stripes attaining anterior margins of fourth and fifth tergites. A pecten of cilia on third tergite of male.

Holotype ♂, allotype ♀, ex lure traps in citrus, Atherton, Queensland, March and April, 1957. (J. Rosser.)

Types in Queensland Museum; holotype Reg. No. T. 5605, allotype Reg. No. T. 5606.

This species closely resembles *A. tigrinus* in general colouration and size but can be separated on the colour of the notopleural callus and infuscation of the *r-m* cross vein. *A. flavinotus, A. javanensis* and *A. trilineatus,* species also possessing three post-sutural vittae, can be separated on general colour and wing and facial markings.

Afrodacus tigrinus May.

This species was described (May 1952) from the female. The male has now been taken and its characters insofar as they differ from those of the female are described below. Certain measurements not included with the description of the female are also given.

Male—Length 4.1 mm.; wing 3.8 mm.

Head.—Vertical length 1.1 mm. *Frons* 1.25 times width. *Antennae*: length of segments: 0.1 mm., 0.2 mm., 0.4 mm.; not reaching edge of hypostome.

Thorax.—Legs (also for female): three pronounced bristles beneath apical half of fore femora. *Wings* covered with microtrichia except alula and outer lower corner of median cell. Proportion of second costal cell to stigma, 0.8:1. Costal band broken beyond stigma; the fulvous basal portion occupying cell R basal to and above median cell, and basal portions of

Fig. 3.

Afrodacus tigrinus. (× 15.)

[*Drawn by William Manley.*

cells R_1 and R_3 below stigma; stigma fuscous; at the wing apex a broad fuscous spot occupying outer portions of cells R_1, R_3 and R_5 and attaining extremity of vein m_{1+2}. *r-m* dividing m_{1+2} in the proportion of 1.2:1. Anal streak absent. Extension of anal cell narrow, partly covered by a pronounced fuscous bulla on the cubital vein; its length in proportion to cu_1+1a, 2:1. A strongly developed supernumerary lobe.

(Note: Proportion of anal cell extension to cu_1 + 1a, for female, is 0.6: 1, not 1.7: 1 (May 1952).)

Abdomen.—Narrower than thorax; bright fulvous tending yellow, except for black markings on tergites as follows:—Paired oval spots on second; laterally on third, fourth and fifth; a narrow triangular medial stripe on fourth and fifth, with apices not attaining anterior margins. A row of cilia on post-lateral margin of third tergite.

Allotype ♂, ex lure traps in mangoes, Kamerunga Experiment Station, Cairns, Queensland, March, 1956. (W. Pont.)

Type in Queensland Museum, Reg. No. T. 5604.

Though resembling *A. furvus* in size, shape and general colouration, it can be separated by the lack of infuscation on *r-m* and the colour of the notopleural callus. The presence of a bulla on the male wing, markings of the wings and face and size readily distinguish it from *A. javanensis* and *A. trilineatus*.

MELANODACUS Perk.

Melanodacus rubidus n.sp.

A moderately large species, with three post-sutural vittae, clear costal cells, small facial spots, black and brown patterned thorax and reddish-brown abdomen covered with yellowish hairs.

Male—Length 7.1 mm.; wing 6.2 mm.

Female—Length, excluding ovipositor, 7.6 mm., wing 7.3 mm.

Head.—Vertical length 1.5 mm. *Frons* flattened, fulvous, except central blotch and area surrounding each *s.or.* bristle fusco-rufous; yellow hairs on vertical calli and along eye margins; length 1.16 times width; bristles: 1 *s.or.*, 3 *i.or.*, lower pairs close together, all strong, black. *Lunule* reddish, shiny. *Antennae* fulvous; length of segments: 0.18 mm., 0.25 mm., 0.8 mm. *Face* fulvous, length 0.5 mm.; a small circular fuscous spot in lower half of antennal groove. *Genae* fulvous with fuscous subocular spot, bristles brownish. *Occiput* fulvous except reddish-brown medially; occipital row weak, black, 3 or 4 each side. *Vertex* fulvous, except reddish around vertical bristles, which are strong, black.

Thorax.—Mesonotum black behind suture; anterior to suture brown except paired black bands commencing in black spot on fore margin and continuing back to suture, also black areas behind humeral calli. *Pleurae* black except red-brown behind humeri and around lower margins of sternopleuron. In addition, yellow as follows:—Humeral and notopleural calli; a pair of broad parallel-sided lateral post-sutural vittae commencing just before suture and ending at upper *p.sa.* bristle; a median post-sutural vitta pointed anteriorly; a broad near parallel-sided mesopleural stripe commencing in wider spot on upper sternopleuron and not attaining humeri; 7/8 upper and 3/4 lower hypopleural calli (remainder black).

Fig. 4.

Melanodacus rubidus. (× 7.5).

[*Drawn by William Manley.*

Postnotum black. *Scutellum* yellow except thin black basal band. *Bristles*: *scp.* 4, *npl.* 2, *mpl.* 1, *a.sa.* 1, *p.sa.* 2, *prsc.* 2, *sc.* 4, all black, *pt.* 1, brown. *Legs* (and coxae) fulvous. *Wings* hyaline except narrow fuscous costal band occupying stigma, cell R_1, outer margin of cell R_3 and ending just beyond end of vein r_{4+5}; and a pale fulvous, short, anal streak. Costal cells hyaline, with microtrichia in outer portion of second cell; proportion of second cell to stigma, 0·7:1. *r-m* dividing m_{1+2} in proportion of 1·3:1. Aggregation of microtrichia at apex of $cu_1 + 1a$, and supernumerary lobe present in male. (Note: Damaged wings of types prevented taking measurements of anal cell extension.)

Abdomen.—Tergites: anterior half of first black, remainder fulvous; anterior third of second black, posterior third whitish, remainder fulvous; third, fourth and fifth fulvous except outer margins of third (and sometimes fourth) fuscous; thin black medial line on fifth; the whole covered with golden hairs. No pecten of cilia on third tergite of male. *Ovipositor* fulvous.

Holotype ♂, allotype ♀, ex collection Department of Agriculture, Port Moresby (Nos. Q3 and Q4). Taken from flowers of *Euphorbia pulcherrima*, Goroka, New Guinea, April, 1955. (J. Szent-Ivany.)

Types in Queensland Museum; holotype Reg. No. T. 5601, allotype Reg. No. T. 5602.

The two other species described under this genus, namely *M. niger* (Tryon) and *M. satanellus* Hering, are small black flies and should not be confused with *M. rubidus*. *Zeugodacus atrisetosus* Perk., known only from the female, is close to *M. rubidus* in many respects but differs in the general colour of the thorax and abdomen and in wing markings.

NEODACUS Perk.

Neodacus lanceolatus Perk.

This species, described by Perkins (1939) from material collected in Papua-New Guinea, has not been recorded previously from Queensland. Specimens were taken in lure traps at Atherton (January, 1956 (J. Rosser)), Cairns (September, 1955, and March, 1956 (W. Pont)) and South Johnstone (July, 1956 and April-May, 1957 (T. Manefield)).

Characters of this material not included in or at variance with the original description are as follows:—

Male—Length 5.2 mm.; wing 4.9 mm.

Female—Length, excluding ovipositor, 6.2 mm.; wing 5.5 mm.

Head.—Vertical length 1.3 mm. *Frons* 1.4 times width. *Antennae* longer than face; short black dorsal bristle on second segment; length of segments: 0.2 mm., 0.2 mm., 0.7 mm. *Face*: length 0.4 mm.; facial spot oval, pointed below.

Thorax.—Wings hyaline except for a narrow fuscous costal band ending midway between r_{4+5} and m_{1+2}, a fuscous anal stripe not attaining wing margin and a narrow infuscation on the outer median cross vein. Costal cells: First pale yellow-brown, second paler to colourless; second costal cell shorter than stigma, the proportion being 0.7:1; microtrichia in upper outer sixth of second cell. *r-m* dividing m_{1+2} in the proportion of 1.4:1. Anal cell extension in proportion to cu_1+1a, 2.7 in male and 1.1 in female.

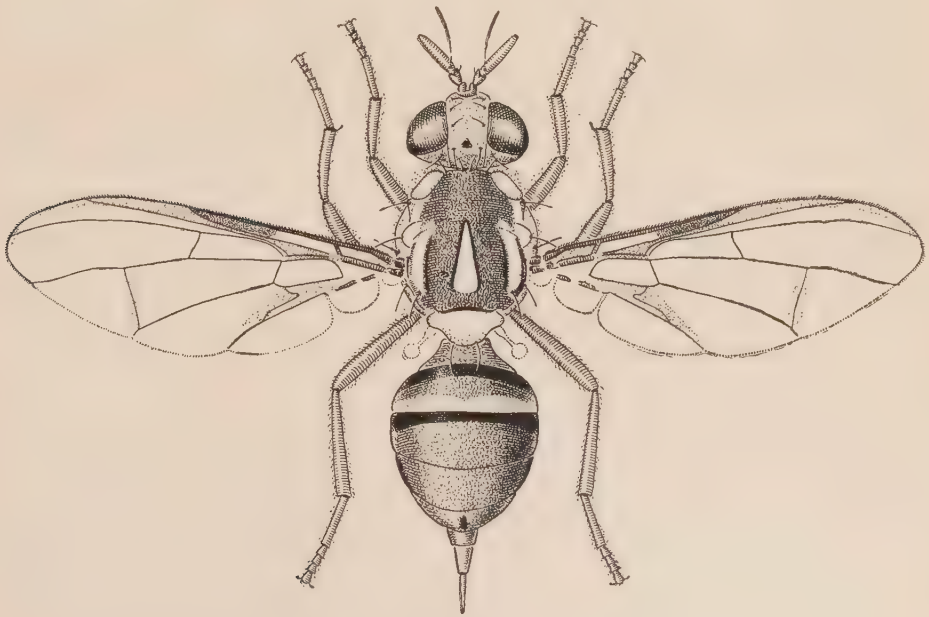

Fig. 5.

Neodacus lanceolatus. (× 9·5).

[*Drawn by William Manley.*

Abdomen.—Pale yellow-brown except:—Dark brown on fore-margin of second, fore-margin of third and extreme lateral margins of third, fourth and fifth tergites; posterior third of second tergite, whitish; pronounced medial black spot on posterior half of fifth tergite. (Note: In some specimens, this black spot is prolonged as a thin medial line extending to anterior edge of fourth tergite.) *Ovipositor* shiny yellow-brown, strongly developed.

Plesiotype ♂ and ♀, ex lure traps in mangoes, Kamerunga Experiment Station, Cairns, Queensland, September, 1955 and March, 1956.

Plesiotypes in Queensland Museum, Reg. Nos. T. 5599 and T. 5600.

PSILODACUS Collart.

Psilodacus exiguus n.sp.

A small species with a slender, elongate-oval abdomen, a narrow wing infuscated along *r-m*, and lacking facial spots and post-sutural lateral yellow stripes.

Male—Length 5.1 mm.; wing 4.4 mm.

Head.—Vertical length 1.2 mm. *Frons* fulvous, 1.4 times width, generally flattened, clothed with fine hairs particularly along eye margins; bristles: 1 *s.or.*, 2 *i.or.* *Antennae* fulvous; length of segments: 0.1 mm., 0.2 mm., 0.6 mm. *Face* shiny, fulvous, covered with fine reddish spots; no facial spot; length 0.45 mm. *Genae* fulvous; bristle brown. *Occiput* fulvous; occipital row weak, pale coloured. *Vertex* not ridged.

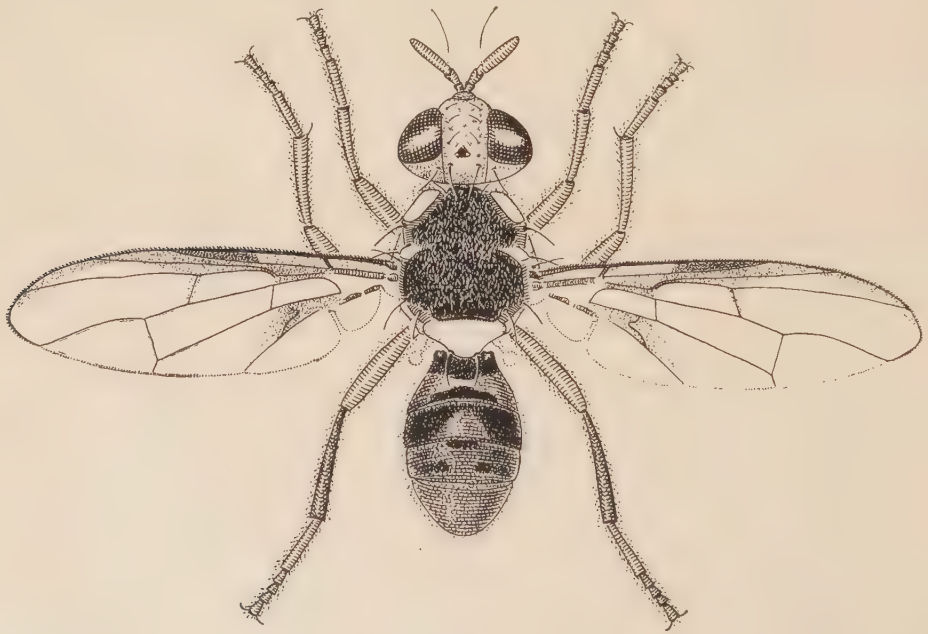

Fig. 6.

Psilodacus exiguus. (× 11.5).

[*Drawn by William Manley.*

Thorax.—Mesonotum black except brown immediately above wing, behind upper *p.sa.* bristles, along inner margin of and immediately behind humeri and extending along fore margin of suture. *Pleurae* brown except black as follows:—A spot above hind coxa, on centre portion of sternopleuron, lower edge of upper and lower 1/6 of lower hypopleural callus. Yellow areas as follows:—Humeral and notopleural calli, and a broadly triangular mesopleural stripe commencing on upper sternopleuron and attaining humeral callus anteriorly. There are no post-sutural vittae. *Scutellum* yellow with a narrow black basal band. *Postnotum* black except central brown line. *Bristles: scp.* 2 (inner pair wanting on specimen), *npl.* 2, *mpl.* 1, *p.sa.* 2, *sc.* 2, all black; *prsc.* and *a.sa.* wanting. *Legs* pale fulvous except hind tibiae slightly brownish. *Wing* narrow, $3\frac{1}{2}$ times as long as wide. Narrow costal band not reaching m_{1+2}. Costal cells fulvous; microtrichia covering outer half of second; proportion of second costal cell to stigma, 0.9:1. *r-m* infuscated, dividing m_{1+2} in the proportion of 2.1:1. Anal cell extension in proportion to cu_1+1a, 0.45:1. Very weak supernumerary lobe.

Abdomen.—Tergites not fused. Narrower than thorax, elongate oval from above. First tergite dark brown, twice as wide as long, antero-lateral humps prominent. Second tergite dark fulvous except fuscous on posterior medial portion. Third tergite fuscous except rich yellow-brown triangular patch medially with base on posterior margin and apex not attaining fore border. Remainder of abdomen shiny, rich yellow-brown with indefinite darker areas on fourth tergite. No pecten of cilia on third tergite.

Holotype ♂, ex lure traps in citrus, Atherton, Queensland, December, 1955. (J. Rosser.)

Type in Queensland Museum, Reg. No. T. 5607.

This species is placed provisionally in *Psilodacus* until further material can be examined. The combination of characters, namely 2 *sc.*, no *a.sa.*, no *prsc.* bristles and third tergite of male not ciliated, fits both this genus and *Leptoxyda* Macquart. The latter monotypic genus, however, was erected to contain *longistylus* Wied., and is separated from *Psilodacus* only by the comparative lengths of the basal segment of the ovipositors, while a truncated fifth abdominal segment in both sexes is characteristic of *longistylus*. The lack of fusion of the abdominal tergites dorsally in *exiguus* does not conform with Collart's (1935) description of *Psilodacus*, a genus confined to the Ethiopian Region. *Daculus* Speiser, represented in Queensland by *D. murrayi* Perk., a large, pale species, possesses 2 *sc.*, no *a.sa.*, no *prsc.* bristles and lacks fused tergites, but possesses cilia on the third tergite of the male.

STRUMETA Walk.

Strumeta hispidula n.sp.

A medium-sized species, generally covered with fine, bristly hairs, with no facial spots, strongly developed antennae, a brown notopleural callus, peculiarly coloured legs, clear costal cells, an orange abdomen, a broadly triangular mesopleural stripe and infuscation of the outer median cross vein.

Female—Length, excluding ovipositor, 5.2 mm.; wing 4.8 mm.

Head.—Vertical length 1.3 mm. *Frons* fulvous, concave, centrally covered with short black hairs; length 1.2 times width; bristles: 1 *s.or.*, 3 *i.or.*, all strong, black. *Antennae* short, fulvous, segments 1 and 2 fringed with short, strong black spines on upper margins;

arista long, strong; length of segments: 0.15 mm., 0.23 mm., 0.67 mm. *Face* pale, fulvous, shiny; no facial spots; length 0.5 mm. *Genae* fulvous, shiny, hoary along eye margin and with several strong black hairs on lower half; bristle strong, black. *Occiput* pale fulvous; occipital row with few strong black hairs on each side, remainder weak. *Vertex* pale fulvous; bristles strong, black.

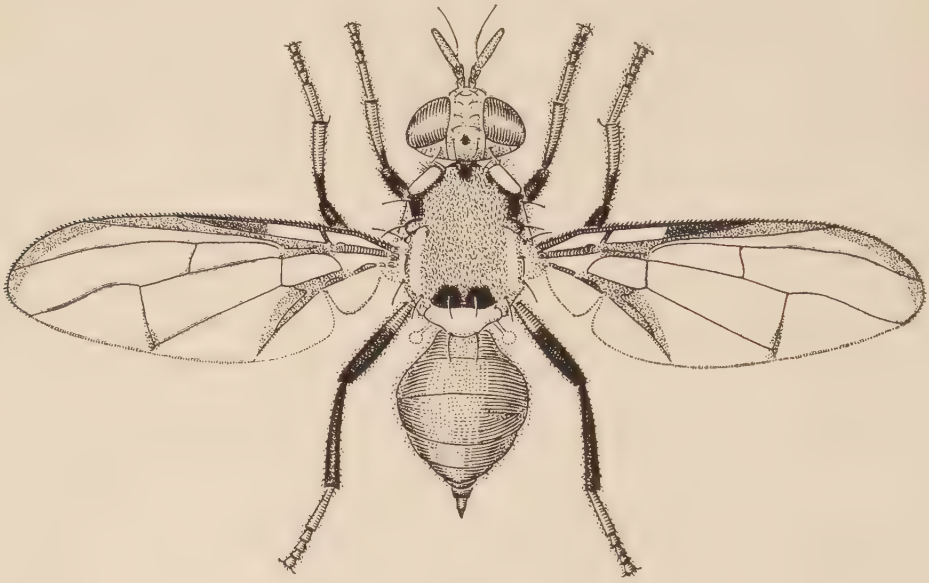

Fig. 7.

Strumeta hispidula. (× 10).

[*Drawn by William Manley.*

Thorax.—Mesonotum yellow-brown except for the following fuscous markings:—Along inner margin of and immediately behind humeri; a central spot on the anterior margin; a pair of almost circular areas at the posterior margin. *Pleurae* fuscous except:—Fulvous immediately below humeri, beneath wings and above middle coxae. With the following yellow markings:—Humeral calli; pair of broad parallel-sided post-sutural lateral stripes ending at the upper *p.sa.* bristles; broad triangular mesopleural stripe with apex at upper margin of sternopleuron and base reaching humeral callus; hypopleural calli (except lower margins fuscous). Notopleural calli fuscous. *Scutellum* yellow except basal margin fuscous. *Postnotum* fuscous except mid-basal portion brown. *Bristles*: *scp.* 4 (inner pair brown), *npl.* 2, *mpl.* 1, *a.sa.* 1, *p.sa.* 2, *sc.* 2 (apical), *pt.* 1, *prsc.* 2, all black. *Legs*: coxae and tarsi fulvous; femora: black except basal quarter of middle and basal third of hind, fulvous; tibiae: fore and middle fulvous, except basal quarter of fore and basal third of middle, black; hind black. *Wings* hyaline, except:—Fuscous band occupying cell R_1, outer margin of cell R_3 and ending midway between veins r_{4+5} and m_{1+2}; a narrow infuscation on posterior half of outer median cross vein; a well defined anal streak not reaching wing margin. Costal cells hyaline; outer half of second covered with microtrichia; second cell shorter than stigma, the proportion being 0.64:1. *r-m* dividing m_{1+2} in proportion of 1.8:1. Proportion of anal cell extension to cu_1+1a is 1.1:1. (Note: A damaged specimen of a male taken in a lure trap at Atherton, March, 1955, shows a well developed supernumerary lobe in the wing.)

Abdomen.—Fulvous tending orange. *Ovipositor:* basal segment shiny, short and flattened, fulvous except darkened apically.

Holotype ♀, ex lure traps in citrus, Atherton, April, 1956. (J. Rosser.)

Type in Queensland Museum, Reg. No. T. 5595.

This species has been placed in *Strumeta* on the assumption that the third tergite of the male abdomen is ciliated. *S. hispidula* can be separated from *S. strigatus* (Perk.), a species also possessing a brown notopleural callus and lacking facial spots, by the colour of the legs, thorax and abdomen and wing markings.

Strumeta mendosa n.sp.

An unusually marked species, lacking post-sutural lateral vittae and with a pronounced black spot on face, coloured costal cells, brown on apex of scutellum, and a parallel-sided mesopleural stripe extending along fore margin of suture.

Female—Length, excluding ovipositor, 7.4 mm.; wing 6.1 mm.

Head.—Vertical length 1.8 mm. *Frons* dull fulvous, flattened; antero-medial raised area not prominent; orbital spots, except upper pair, small; length, 1.4 times width; vertical calli shiny, covered with short, pale hairs; bristles: 1 *s.or.*, 3 *i.or.*, middle pair very close to lower pair. *Antennae:* segments 3 missing, remainder fulvous. *Face* shiny, fulvous, length 0.4 mm.; facial spot large, oval, confluent with inner margin of antennal groove but not reaching hypostome. *Genae* fulvous, with red-brown subocular mark; bristle strong, pale. *Occiput* fulvous; occipital row strong, black, five on each side. *Vertex* fulvous, not ridged; bristles strong, black.

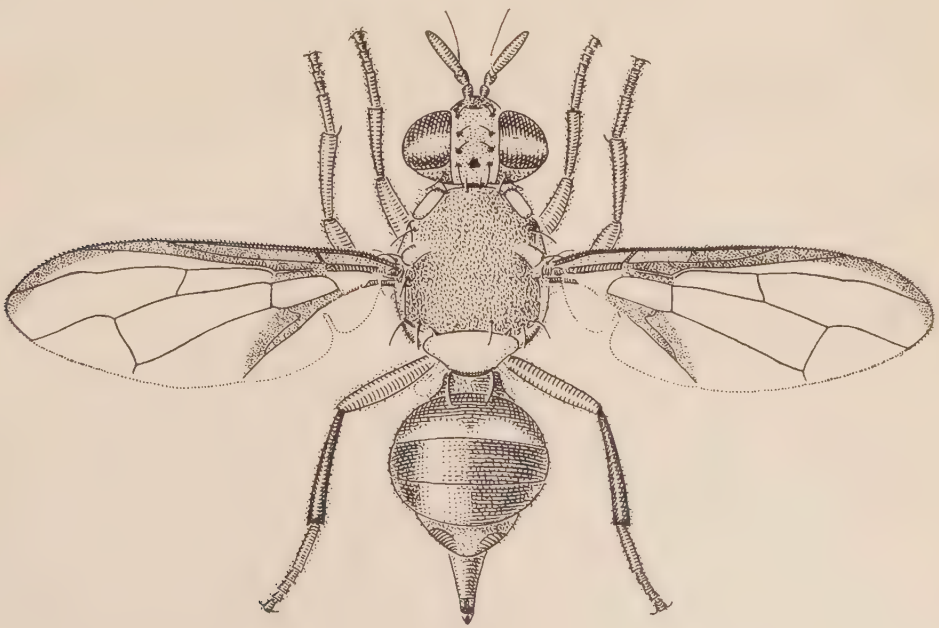

Fig. 8.

Strumeta mendosa. (× 8).

[*Drawn by William Munley.*

Thorax.—Pale reddish-brown except fuscous immediately behind wing, along fore and lower margins of sternopleuron and immediately above hind coxae. With the following yellow markings:—Humeral and notopleural calli; an almost parallel-sided mesopleural stripe commencing at upper margin of sternopleuron, enclosing notopleural callus and ending in a point a short distance along suture; 5/6 of upper and 3/4 of lower hypopleural calli (remainder dark brown). *Scutellum* yellow except brown at apex between bristles. *Postnotum* dark red-brown. *Bristles: scp.* 4, *npl.* 2, *mpl.* 1, *a.sa.* 1 (pale, reclinate), *p.sa.* 2, *sc.* 2, *pt.* 1, *prsc.* 2 (Note: Only the right side bristle present.) *Legs* fulvous, except hind femora fuscous; coxae dark fulvous, fore pair paler. *Wings* hyaline, except:—Fulvous costal cells; a fuscous costal band occupying all cell R_1, outer portion of cell R_3 and ending midway between veins r_{4+5} and m_{1+2}; a clearly defined anal streak almost reaching wing margin. Microtrichia covering whole of second and anterior half of first costal cells. Second costal cell shorter than stigma, the proportion being 0.7:1. *r-m* dividing m_{1+2} in the proportion of 1.4:1. Proportion of the anal cell extension to cu_1+1a, 1.5:1.

Abdomen.—As broad as thorax, almost circular in outline. Tergites 1 and 2 brown except pale fulvous to whitish on posterior half of latter. Remainder fulvous tending brownish on anterior half of 3 and laterally on 3, 4 and 5; pale fulvous between large shining fulvous areas on 5. *Ovipositor:* basal segment twice as long as fifth tergite measured dorsally, shiny, testaceous, strongly developed, conical, slightly compressed dorso-ventrally.

Holotype ♀, ex lure traps in citrus, Atherton, June, 1956. (J. Rosser.)

Type in Queensland Museum, Reg. No. T. 5594.

This species has been placed provisionally in *Strumeta* on the assumption that the *prsc.* bristles are normally present and the third tergite of the male is ciliated. The absence of the post-sutural lateral vittae and the extension of the mesopleural stripe on to the mesonotum show affinity with some Queensland *Neodacus,* a genus lacking *prsc.* bristles but possessing a pecten of cilia on the third tergite of the male. The combination of characters given above, however, enables the separation of *S. mendosa* from all known species of *Strumeta* and *Neodacus.*

Strumeta notatagena May.

This species was described (May 1952) from the female. The characters of the male insofar as they differ from those of the female, and measurements not included with the earlier description, are as follows:—

Male—Length 5.4 mm.; wing 4.6 mm.

Head.—Vertical length 1.3 mm. *Frons* with central dark brown blotch connected to ocellar triangle by thin brown line and widened laterally to connect with central pair of orbital spots. *Antennae* strongly elbowed and held well away from face; length of segments: 0.2 mm., 0.3 mm., 0.7 mm.; segment 2 with strong black bristle. *Face:* length 0.4 mm.

Thorax.—As for female, except:—*Legs* brown towards apex of hind femora. *Wings*: costal cell shorter than stigma, the proportion being 0.7:1; length of anal cell extension to cu_1+1a in proportion of 2.1:1. A distinct but poorly developed supernumerary lobe present. Long black shaggy hairs covering most of anal cell extension and dense aggregation of microtrichia at inner extremity of cu_1+1a.

Abdomen.—Narrow and elongate. Third tergite ciliated.

Allotype ♂, 2 ♂♂ paratypes, ex lure traps in citrus, Atherton, Queensland, March, 1956. (J. Rosser.)

Types in Queensland Museum; allotype Reg. No. T. 5596, paratypes Reg. Nos. T. 5597 and T. 5598.

Strumeta robiginosa n.sp.

A medium-sized to large species, rusty brown in general colour, with fulvous costal cells, narrow costal band, broad parallel-sided lateral post-sutural stripes and abdomen wholly yellow-brown except for pronounced fuscous spots on fifth tergite.

Female—Length, excluding ovipositor, 7.4 mm.; wing 6.6 mm.

Head.—Vertical length 1.8 mm. *Frons* fulvous, flattened on posterior half; a well-defined medial hump, clothed with short black hairs on anterior half; length 1.4 times width; bristles: 1 *s.or*, 2 *i.or.*, brown. *Antennae* fulvous; length of segments: 0.3 mm., 0.4 mm., 0.8 mm. *Face* shiny, fulvous; length 0.6 mm.; a circular fuscous spot on inner half of antennal groove just below the mid-point. *Genae* fulvous with subocular spot; bristle brown. *Occiput* fulvous; occipital row strong, brown, approximately eight each side. *Vertex* fulvous; bristles brown.

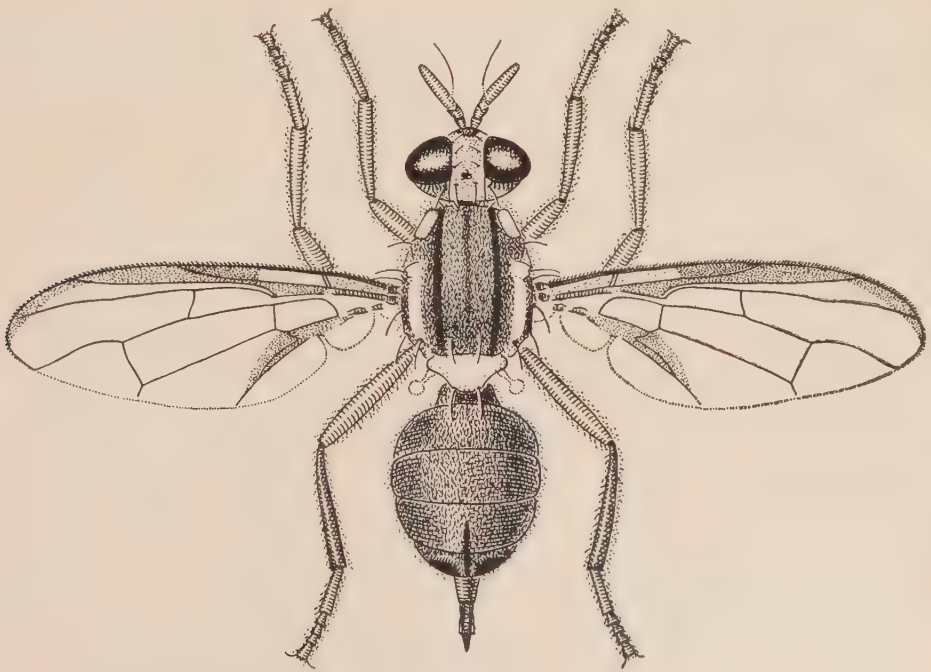

Fig. 9.

Strumeta robiginosa. (× 8.)

[*Drawn by William Manley.*

Thorax.—Pale red-brown except for two darker narrow bands commencing at inner edge of humeri and extending to scutellum; covered with short golden hairs. Yellow markings as follows:—Humeral and notopleural calli; broad parallel-sided lateral post-sutural stripes, narrowing posteriorly and attaining *p.sa.* bristle; mesopleural stripe commencing as a well-defined spot on upper edge of sternopleuron and broadening to almost reach humeral callus anteriorly; most of upper and 5/6 lower hypopleural calli (remainder brown). Hoariness on anterior margin of mesopleuron and most of sternopleuron. *Scutellum* yellow. *Postnotum* dark brown. *Bristles: scp.* 4, *npl.* 2, *mpl.* 1, *a.sa.* 1, *p.sa.* 2, *sc.* 2, *prsc.* 2, *pt.* 1, all brown. *Legs* fulvous, hind tibiae slightly darker. *Wings* hyaline except fulvous costal band and anal stripe. Costal band occupying cell R_1, outer margin of cell R_3 and terminating just beyond end of r_{4+5}. Costal cells pale fulvous (second cell almost colourless); microtrichia covering upper outer fifth of second cell; proportion of second cell to stigma 0.7:1. *r-m* dividing m_{1+2} in proportion of 1.4:1. Proportion of anal cell extension to cu_1+1a, 1.14:1.

Abdomen.—Wholly rusty yellow-brown except for large fuscous shining spots on fifth tergite and a thin fuscous medial line extending from mid-point of fourth to posterior margin of fifth tergites. The whole covered with short, appressed golden hairs. *Ovipositor* sturdy, shiny.

Holotype ♀, 1 ♀ paratype ex lure traps in citrus, Stoney Creek, Cairns district, Queensland, November, 1955. (W. Pont.)

Types in Queensland Museum; holotype Reg. No. T. 5592, paratype Reg. No. T. 5593.

Two other Queensland species, *S. fagraea* (Tryon) and *S. mutabilis* May, are somewhat similarly coloured. The former is separated by having a longer ovipositor and narrower mesopleural stripes. The latter has black areas on the fore margin of the mesonotum and no facial spots. Both *S. barringtoniae* (Tryon) and *S. breviaculeus* Hardy differ in the colouration of the abdomen. *S. latifrons* (Hendel), though having an entirely rufous abdomen, differs from *S. robiginosa* in the colouration of the thorax and having an apical spot on the wing.

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TECHNICAL NOTE.

ROOT-KNOT NEMATODE CONTROL IN TOBACCO SEEDBEDS.

The root-knot nematode *Meloidogyne javanica* (Treub) Chitwood is a serious pest of tobacco in Queensland. Smith (1957) has shown that the use of tobacco seedlings with trace infestations of root-knot nematode can cause serious crop losses, and a high level of control in the seedbed is therefore essential.

For many years effective seedbed sterilization has been obtained by burning either antbed (the inner woody material of termite mounds) or wood. The shortage of these materials in some areas has led to a demand for chemicals which will control both nematodes and weeds without retarding the growth of tobacco seedlings. Haney (1950) compared the use of DD, "Cyanamid," a "Cyanamid"-urea mixture and termite material. Since that paper EDB has come into widespread use for nematode control in field crops, methyl bromide has been employed in small quantities for nematode and weed control in seedbeds, and experimental supplies of "Vapam 4-S" (3 lb. sodium N-methyl dithiocarbamate per 40 U.S. gal. water) have become available.

The two trials reported in this note were set out in the Lower Burdekin district during 1956, as randomised blocks with 4 replicates and a plot size of 16 sq. ft., to test the value of these materials for tobacco seedbeds. The EDB used was made from a concentrate with Sp.G. $\frac{25}{25}$ 2·170.

Trial 1.

This trial, on a fine sandy loam at Clare, was seeded on Aug. 23–24 and benzol treatments (see Pont 1956) were commenced four weeks later. Weed counts in each plot were made weekly for three weeks by taking four 6 in. square quadrats at random within 2 ft. x 3 ft. datum areas. On Oct. 16, seedlings from six 8 in. square quadrats within the datum areas were pulled, cleaned and weighed, and examined for nematode infestations.

The treatments were:—

(1) Antbed closely packed 6 in. high, and burnt on Aug. 22.

(2) EDB 12½ per cent. v/v injected 6 in. deep at 9 in. spacings at the rate of 2 ml. per hole on Aug. 8.

(3) Methyl bromide; 2 x 20 ml. ampoules per plot (i.e., at the rate of 95 lb./100 sq. ft.) applied under plastic covers on Aug. 21. The covers were removed after 24 hours.

(4) Treatments 2 and 3 combined.

(5) Control.

The results are given in Table 1.

Table 1.

RESULTS FROM TRIAL 1, CLARE.

Treatment.	Weeds per Datum Area (mean number).	Green Weight of Seedlings (g./quad.).	Percentage of Seedlings Nematode Infested.
Antbed	2	350·74	0
EDB	400	71·57	0
Methyl bromide	27	262·4	0
EDB + Methyl bromide	20	305·016	0
Control	532	52·82	35·2
Differences necessary for significance 5%	..	95·26	..
Differences necessary for significance 1%	..	67·59	..

Trial 2.

The methods used in this trial, which was set out on a sandy loam at Rita Is., were similar to those in the Clare trial. Seedlings were pulled on Dec. 28.

The treatments were:—

(1) Wood 1–2 in. in diameter closely stacked 2 ft. 6 in. high, and burnt on Nov. 10.

(2) EDB 12½ per cent. v/v injected 6 in. deep at 9 in. spacings on Oct. 16, 2 ml. per injection.

(3) Methyl bromide, 2 x 20 ml. ampoules per plot applied under plastic covers on Nov. 8. The covers were removed after 24 hours.

(4) Treatments 2 and 3 combined.

(5) "Vapam 4-S" applied at the rate of 2 quarts per 100 sq. ft. (i.e., 364 ml. per plot) on Oct. 19.

The results are given in Table 2.

Table 2.

RESULTS FROM TRIAL 2, RITA ISLAND.

Treatment.	Weeds per Datum Area (mean number).	Green Weight of Seedlings (g./quad.).	Percentage of Seedlings Nematode Infested.
Burnt timber	24	397	7·3
EDB	192	91	4·2
Methyl bromide	60	290	1·3
EDB + methyl bromide	60	260	·1
"Vapam 4–S"	60	292	40·7
Control	172	46	46·3
Differences necessary for significance 5%	..	127	..
Differences necessary for significance 1%	..	92	..

The results from these trials show that sterilization by burning either antbed or wood is still the most effective method of seedbed treatment for tobacco. Methyl bromide fumigation, which gave good control of both nematodes and weeds, may prove a satisfactory substitute. The seedlings from methyl bromide treated plots, however, were inferior in size to those from the burnt plots. The economic significance of this difference is now being studied in related seedbed and field trials.

EDB at the rate used controlled nematodes but not weeds.

"Vapam 4-S" gave satisfactory weed control but did not control nematodes.

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ERRATA.

"A Survey of the Incidence of Copper Deficiency in Dairy Cattle in Coastal Queensland South of Brisbane."

Owing to misinterpretation of the legend of a map, an error appeared in the above paper published in Vol. 14, No. 1 of this Journal.

The first paragraph under "IV. Discussion" on page 25 should read as follows:—

"Skerman (1952) mapped the alluvia of streams in south-eastern Queensland. These are shown as rising in the tertiary basalts of the hinterland but the alluvium is highly siliceous, being derived mainly from old metamorphics, mesozoic sediments of sandstone type, or redistributed marine sands of recent age. These soils are periodically inundated by floods and drainage is a serious problem. Due to their origin they are generally of low fertility and acid to strongly acid in reaction. This, together with poor pasture management, has led to the invasion of sown pastures of paspalum (*Paspalum dilatatum*) and white clover (*Trifolium repens*) by narrow-leaved carpet grass (*Axonopus affinis*). White clover has persisted rather more than paspalum, but both are now dominated by the more aggressive carpet grass. This is particularly true in the alluvial areas towards the mouths of rivers, where drainage is a major problem.

"Toxicity of the Leaves of Macrozamia spp. for Cattle."

Line 27 on page 51 of Vol. 14, No. 2, of this Journal should read:

"reported here the horn was not affected, and it is thought that in the field damage".

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